UNIVERSITY OF ALBERTA

Immediate and long-term effects of transluminal balloon angioplasty in a new canine carotid artery model of vasospasm

by

Joseph Frank Megyesi ©

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**

in

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled IMMEDIATE AND LONG-TERM EFFECTS OF TRANSLUMINAL BALLOON ANGIOPLASTY IN A NEW CANINE CAROTID ARTERY MODEL OF VASOSPASM, submitted by Joseph Frank Megyesi in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Experimental Surgery.

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To my parents, Irene and Ted.

Because, no man is an island.

John Donne

ABSTRACT

Despite an improved understanding of its pathophysiology, cerebral vasospasm following aneurysmal subarachnoid hemorrhage (SAH) remains a serious problem. A newer strategy to treat vasospasm is transluminal balloon angioplasty (TBA), in which a small inflatable balloon attached to the tip of an endovascularly placed catheter is navigated into the narrowed artery in order to dilate it. The technique has proven clinically successful but only recently has the underlying mechanism been studied.

One of the difficulties in studying TBA is the lack of a suitable model of vasospasm which uses arteries approximating the size of those found at the base of the human brain. In the first part of this study a new model of vasospasm using the canine high cervical internal carotid artery (ICA) was developed. In 8 dogs placement of a blood clot-filled silicone elastomer cuff around the ICA resulted in angiographic arterial narrowing in 7 days. Arteries had pharmacological and morphological (electron microscopic) characteristics consistent with vasospasm. Placement of an empty cuff around the ICA had no significant effect on the artery. Since the canine ICA is approximately the same size as the human intracranial arteries commonly affected by vasospasm (the internal carotid artery or M1 segment of the middle cerebral artery), and is suitable for endovascular manipulation, this new model of vasospasm was used for further experiments to study the mechanism of action of TBA.

In the next study, 12 dogs underwent exposure of both ICAs and TBA of one ICA. Both ICAs were then surrounded with blood clot-filled silicone elastomer cuffs. After 7 days undilated arteries developed angiographic vasospasm, with characteristic morphological and pharmacological features. Arteries subjected to TBA remained angiographically dilated after 7 days despite the presence of a surrounding blood clot. The arteries exhibited altered smooth muscle cell function as determined by pharmacological analysis and some morphological changes, but no frank vessel wall disruptions. The results supported the concepts that TBA acts mainly by causing a functional alteration of the vessel wall and that early TBA might be optimal in the treatment of vasospasm.

The next study looked at the long-term effects of TBA. Forty dogs were used. Blood clot placement was performed on day 0, TBA performed on day 7 and arteries studied angiographically, pharmacologically and morphologically on days 7, 14, 21, 28, and 56. Arteries surrounded by an empty cuff behaved like normal arteries on all study days. Arteries surrounded by a blood filled cuff exhibited angiographic vasospasm on days 7 and 14 with resolution by day 21. Pharmacological and morphological features characteristic of vasospasm were present in these arteries. Vasospastic arteries undergoing TBA showed an immediate angiographic dilatation that was durable and a functional impairment of the vessel wall on days 7, 14 and 21, but not on days 28 and 56. Morphological alterations were present on days 7 and 14, resolving by days 21 and 28, and almost absent by day 56. Normal arteries undergoing TBA showed an immediate angiographic dilatation and a functional impairment of the vessel wall on days 7 and 14, but not on days 21, 28 and 56. Morphological alterations were present on day 7, resolving by day 14, and almost absent by days 21, 28 and 56. It was concluded that the functional and morphological alterations caused by TBA are not permanent.

Key Words: Dog, Subarachnoid Hemorrhage, Vasospasm
Transluminal Balloon Angioplasty

PREFACE

The first chapter of this work reviews the pathogenesis and treatment of cerebral vasospasm. Topics covered include the definition vasospasm, its clinical relevance, the structure of cerebral blood vessels, the mechanisms of smooth muscle contraction, the pathophysiological theories used to explain vasospasm, morphological and pharmacological changes seen in vasospastic arteries, and the prophylaxis and treatment of vasospasm, with special attention to transluminal balloon angioplasty. The remainder of the thesis deals with studies performed using a new canine high cervical internal carotid artery model of vasospasm developed in the Cerebrovascular Research Laboratory at the University of Alberta. The second chapter begins by reviewing existing *in vivo* animal models of subarachnoid hemorrhage using both intracranial and extracranial arteries. Various techniques to simulate subarachnoid hemorrhage and vasospasm are presented. The need for an *in vivo* animal model of vasospasm in which transluminal balloon angioplasty can be readily performed is explained. Chapter two contains the first experiments performed in the establishment of the canine high cervical internal carotid artery model of vasospasm. The third chapter deals with the ability of transluminal balloon angioplasty, performed prior to the onset of vasospasm, to prevent vasospasm development in the canine high cervical internal carotid artery model. The pharmacological and morphological effects of this technique on the vessel wall are presented. The fourth chapter describes studies used to determine the immediate and long-term effects of transluminal balloon angioplasty on established vasospasm in the new model. The time course of the pharmacological and morphological alterations seen with transluminal balloon angioplasty are presented. This ordering represents the sequence in which these studies were performed. The final chapter reviews possible explanations for the study results, suggests further experiments, and comments on potential clinical implications of the results for the treatment and prophylaxis of vasospasm seen in humans following aneurysmal subarachnoid hemorrhage.

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CHAPTER ONE
CEREBRAL VASOSPASM: PATHOGENESIS AND TREATMENT

Cerebral vasospasm: definition and relevance of the problem

Cerebral vasospasm is delayed-onset, prolonged and pathological cerebral arterial narrowing following aneurysmal subarachnoid hemorrhage (SAH) (1,2). The diagnosis of symptomatic cerebral vasospasm should be reserved for patients with delayed-onset focal and/or global neurological deterioration (2). Even in these cases other causes of neurological worsening should be ruled out and arterial narrowing should be confirmed by imaging studies.

Ecker and Riemenschneider (3) were the first to report angiographic vasospasm following aneurysmal SAH in 1951. The delayed type of vasospasm which is important clinically in humans is usually evident angiographically in the first to second week after SAH (1). Vasospasm affects primarily large conducting arteries which course through the subarachnoid cisterns on the basal aspect of the brain. These are the arteries upon which saccular aneurysms arise and consequently those which are encased with thick blood clot after aneurysmal bleeding. This type of vasospasm should not be confused with the immediate but transient narrowing of cerebral arteries seen immediately after SAH in some animals. Furthermore, brief arterial narrowing of less than 30 minutes can be observed after mechanical irritation of cerebral arteries in animals (4) and is sometimes noted during intracranial surgery in humans. However, Grote and Hassler (5) did not notice vasospasm in three patients whose aneurysms bled during angiography and Weir et al. (1) did not find angiographic vasospasm in SAH patients within 24 hours of hemorrhage.

A number of studies have looked at the incidence and prevalence of vasospasm (6-10). The majority of patients with aneurysmal SAH develop some degree of angiographic cerebral vasospasm, but less than one third of these patients develop symptomatic cerebral ischemia. Furthermore, in current neurosurgical practice, the incidence of patients with poor-outcome secondary to vasospasm is less than 10% (2). Nevertheless, cerebral vasospasm remains the largest cause of preventable morbidity and

mortality in survivors of aneurysmal SAH (2).

The pathogenesis of cerebral vasospasm has undergone extensive study over the past three decades, with two main schools of thought emerging. Some feel that prolonged, pathological smooth muscle cell contraction is responsible for cerebral vasospasm (11-15), while others think that luminal narrowing is more likely due to wall contraction arising from proliferation of fibroblasts and increased extracellular fibrous protein matrix (16-21). Many favor a combination of the two ideas. Both concepts will be reviewed in some detail below.

The management of cerebral vasospasm has improved markedly over the past three decades. This will also be reviewed, with particular emphasis on a new and increasingly popular technique called transluminal balloon angioplasty (TBA), in which vasospastic arteries are dilated with an inflatable balloon attached to an arterial catheter (22-24).

Some of the concepts regarding cerebral vasospasm are better appreciated with some knowledge of the morphology of normal cerebral arteries and the mechanisms of contraction and relaxation that occur in the smooth muscle cells that exist in the walls of those arteries. A good general review of arterial morphology is found in Simionescu and Simionescu (25) while cerebral arteries in particular are described in Dahl (26). Reviews of smooth muscle contraction and relaxation may be found in papers by Walsh (27,28). Following is a brief summary of both topics.

Structure of normal cerebral arteries

There are three basic structural constituents that make up the wall of blood vessels: the endothelium (endothelial cells), the muscular tissue (smooth muscle cells), and the connective tissue that includes the elastic elements (25). Throughout the circulatory system these tissues are unevenly distributed in the vessel wall.

Endothelial cells. The vascular endothelium is the sheet of thin squamous epithelial cells that lines blood vessels (25). The endothelium rests on a basal lamina (basement membrane) that varies in thickness and continuity. The endothelium and its basal lamina form the main permeability barrier and regional differences exist that create

variably porous vessel walls. The endothelium may be partially surrounded by pericytes which are cells that perform variable functions which may include contraction and phagocytosis. In vessels where smooth muscle cells occur near the endothelium there are myoendothelial junctions.

Smooth muscle cells. Smooth muscle cells in the wall of blood vessels appear either as organized concentric layers helically arranged (as found in larger arteries) or as longitudinally occurring bundles of muscle cells intermingling with other vessel wall structures (25).

Connective tissue. Connective tissue is composed of collagens, proteoglycans, fibronectin, fixed and wandering cells, and ground substance (25). It is found largely in the subendothelial layers and on the outer aspect of the blood vessel in the adventitial layer. Small bundles of smooth muscle may intercalate with these accumulations of connective tissue. There are two types of elastic elements: isolated elastic fibers that are dispersed throughout the vessel wall and elastic sheets that may be organized as separate units called the internal elastic lamina (IEL) and the external elastic lamina (EEL).

Organization within blood vessels. The entire vascular system is organized with the tissues described above arranged in concentric layers (25). Although regional variations exist the general plan is one of three layers or tunics. From the lumen outward these tunics are: (1) the tunica intima which contains the endothelium, the basal lamina, the subendothelial connective tissue, and the internal elastic lamina; (2) the tunica media which contains the muscular cells, elastic lamellae, and external elastic lamina; and (3) the tunica adventitia that contains the connective tissue and its various components. Cerebral vessels can be divided into five principal groups: arteries, arterioles, veins, venules, and capillaries. The large intracranial arteries (where cerebral vasospasm occurs) as well as the small extracerebral arteries are composed of a tunica intima, tunica media, and tunica adventitia (26), comprising 17%, 52%, and 31% of the arterial wall, respectively (29).

Tunica intima. The arterial lumen is lined by a single layer of endothelial cells without fenestrations. Uninterrupted tight junctions between endothelial cells form an effective obstacle to vascular diffusion (30), a component of the so-called blood-brain

barrier. Endothelial cells have comparatively high numbers of mitochondria, hence their high metabolic rate and functional importance (31). Endothelial cells have been shown to have actin- and myosin-like filaments in the cytoplasm (32-34). These myofilaments may enable endothelial cells to contract, potentially contributing to arterial lumen narrowing in vasospasm. The internal elastic lamina in the subendothelial space is made up of a homogenous matrix, or ground substance, in which the elastic tissue is deposited (31). This ground substance is in close apposition to cell membranes of the overlapping endothelium without any intervening gap. The elastic tissue is concentrated into a single band and borders on the tunica media.

Tunica media. The tunica media of cerebral arteries and arterioles is comprised of smooth muscle that makes up 72% of the layer by mass. The cells are arranged circumferentially, except around major bifurcations where there is multilayering in different directions (26,35). Adjacent smooth muscle cells are often separated by a single shared basement membrane (35). The basement membrane forms the framework of the tunica media by surrounding all smooth muscle cells and providing a base for the endothelium on the luminal side and for the adventitia on the outside. This frame effectively links cells to each other and allows the vessel wall to function as an integrated unit. Smooth muscle cells located in the walls of muscular arteries contain small areas of conspicuous dense material that lie underneath the cell surface. These dense bodies are believed to be attachment devices, anchoring the system of myofilaments to the cell surface at defined points (35). Collagen fibers found in the tunica media (and tunica adventitia) are arranged parallel to the axis of the artery and hence perpendicular to smooth muscle cells (26,35).

Tunica adventitia. The tunica adventitia of cerebral arteries is comprised of bundles of collagen which are arranged longitudinally or circumferentially. The outer border of the vessel wall is well defined, being made up of thin cellular processes which originate from spindle-shaped elongated cells possessing the fine structural features of fibrocytes (26). Where the arteries enter the cerebral cortex there is a membranous junction between the vessel wall and the cytoplasmic processes of the pial arachnoid cells (26).

Innervation of cerebral arteries. Larger intracranial extracerebral arteries are accompanied by periadventitial nerves separated from the outer coat of the vessel wall (26,35). Segmental branches enter the tunica adventitia at regular intervals, become devoid of a perineurium and arborize, but do not enter the tunica media. Myelinated and unmyelinated fibers are found in larger arteries; only unmyelinated fibers are found in arterioles. Membranous neuromuscular junctions are not observed (26).

Extracellular matrix. In cerebral arteries the extracellular matrix or connective tissue of the vessel wall consists of collagens, elastins, glycosaminoglycans, proteoglycans, and noncollagenous glycoproteins. The extracellular matrix is important for cell growth and development as well as cell migration and response to injury (36,37). All collagen types (I,II,IV,V,VI, and VIII) present in systemic arteries can be found in cerebral arteries (36-38). Types I and II are fibril-forming interstitial collagens that comprise 80-90% of total arterial collagen; they are found in all three arterial layers (37). Type IV collagen is located in basement membrane (39), which also contains laminin, heparan sulfate proteoglycan and entactin. Locations and functions of the remaining three types of collagen (V, VI, and VIII) are uncertain.

Differences between cerebral arteries and systemic arteries. Cerebral arteries have a thinner wall than systemic arteries of the same caliber, especially the tunica media and tunica adventitia, possibly due to a dampening of the arterial pressure pulse by the surrounding cerebrospinal fluid (40). The muscular coat of small pial arterioles is only a single layer of muscle cells. Electron microscopy has demonstrated ultrastructural differences between cerebral arteries and systemic arteries (26,35,40): (1) extracranial arteries have both external and internal elastic laminae while cerebral arteries have only an internal elastic lamina; (2) extracranial arteries are strongest at the tunica adventitia while cerebral arteries are strongest at the internal elastic lamina and tunica media; (3) the collagen structure of cerebral arteries often differs from that of systemic muscular arteries of a similar size; and (4) most extracranial arteries receive nutrient supply from vasa vasorum while only large proximal cerebral arteries are supplied in this manner. Distal cerebral arteries may be nourished from the cerebrospinal fluid (CSF) via "rete vasorum" which are endothelium-lined intraadventitial channels connected to the

subarachnoid space via stomata. The large arteries at the skull base course through subarachnoid cisterns and are suspended from the brain by many arachnoid trabeculae. The more distal penetrating intracerebral arterioles are surrounded by CSF in so-called Virchow-Robin spaces, which do not extend to the capillary bed where the blood-brain barrier exists. The lack of surrounding soft tissues and the relative thinness of the arterial wall (compared to walls of systemic arteries) may be predisposing factors for the formation and rupture of aneurysms located on arteries at base of the brain. The presence of basal cisterns in which blood can accumulate and the lack of readily available phagocytic cells to remove blood breakdown products may be reasons why symptomatic vasospasm develops in cerebral but not systemic arteries.

Smooth muscle contraction

Contractile proteins in smooth muscle

Smooth muscle cells consist of: (1) thin filaments, composed mainly of actin monomers in double helical strands, and associated with tropomyosin arranged along the length of actin filaments; (2) intermediate filaments, composed of desmin or vimentin; and (3) thick filaments, composed of aggregated myosin molecules. Thick and thin filaments generate contraction via cross-bridge cycling, in accordance with the sliding filament model of muscle contraction: contraction occurs by a relative sliding of actin and myosin filaments, without actual shortening of the filaments (41). Intermediate filaments form a network connecting force-generating units in the cell via the dense bodies and hence distribute tension throughout the cell.

In smooth muscle cells actin filaments are attached to dense bodies. While one end of an actin filament is attached to either a cytoplasmic or a membrane-associated dense body, the other end of the actin filament interdigitates with myosin filaments. In turn, the opposite ends of these myosin filaments overlap the free ends of another set of actin filaments, the opposite ends of which are associated with another dense body. Upon contraction associated dense bodies will be pulled toward the center of the cell. Two isoforms of actin filament exist in smooth muscle: the smooth a, which predominates in vascular smooth muscle, and the smooth g. Tropomyosin is present in

smooth muscle and is located in the grooves between the two strands of the actin double helix. In striated muscle, tropomyosin in conjunction with troponin helps regulate contraction; however, lack of troponin in smooth muscle makes the role of tropomyosin uncertain in these cells. Smooth muscle myosin is a hexamer with two heavy chains and two pairs of light chains (42). Each myosin molecule consists of a long, rodlike tail and two globular heads, each with an actin-binding site and a site of ATP hydrolysis.

Regulation of smooth muscle contraction by calcium

Calcium homeostasis. Smooth muscle cell contraction is regulated primarily by the concentration of free cytosolic (sarcoplasmic) calcium ($[Ca^{2+}]$). In resting smooth muscle cells $[Ca^{2+}]$ is 120-270 nM (43). When the smooth muscle cell is stimulated $[Ca^{2+}]$ increases transiently to 500-700 nM (44). The sources of calcium are the extracellular space and the lumen of the sarcoplasmic reticulum (SR) (45), where the calcium concentration is in the millimolar range. Sarcoplasmic free $[Ca^{2+}]$ is regulated by movement of calcium across the sarcolemmal and SR membranes. There are two major mechanisms for entry of extracellular calcium: electromechanical coupling (voltage-gated calcium channels) and pharmacomechanical coupling (receptor-operated calcium channels) (46).

Electromechanical coupling in smooth muscle (Figure I-1). Membrane depolarization (caused by agents such as potassium chloride [KCl]) opens calcium channels and allows extracellular calcium to diffuse down its concentration gradient into the sarcoplasm. Calcium influx stimulates ryanodine receptors which in turn trigger intracellular calcium release through "calcium induced calcium release" mechanisms. This leads to myosin phosphorylation as described below.

Pharmacomechanical coupling in smooth muscle (Figure I-2). Ligands such as noradrenalin, serotonin, prostaglandin F2α, as well as certain hormones act on specific receptors on the sarcolemmal membrane that are coupled via a GTP-binding protein (G-protein) to the enzyme phophoinositide-specific phospholipase C (PLC) (46). Binding of ligands to the receptors activates phospholipase C to hydrolyze membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP_2), thus generating two second messengers: the

lipid 1,2-diacylglycerol (DAG) and water-soluble inositol 1,4,5-trisphosphate (IP_3) (47). DAG accumulation is biphasic: hydrolysis of PIP_2 produces only a transient rise in DAG, followed by a more sustained accumulation of DAG from phosphatidylcholine (PC) degradation by PC-specific phospholipase C, or concerted actions of phospholipase D and phosphatidate phosphohydrolase (48). DAG activates sarcolemmal membrane-associated protein kinase C (PKC), while IP_3 diffuses across the sarcoplasm to interact with IP_3 receptors on the SR membranes (49) (this is illustrated on the left side of Figure I-5, but in the context of cerebral vasospasm). The IP_3 receptors on the membranes of SR are also calcium channels and they mediate calcium flow from the lumen of the SR into the sarcoplasm (49). The increased $[Ca^{2+}]$ leads to myosin phosphorylation as described below.

Restoration of calcium concentration to resting-state. After contraction calcium is removed from the sarcoplasm to restore the resting-state calcium concentration and to maintain homeostasis thereafter (50). Calcium pumps in the sarcolemma and the SR membrane are calcium-transporting ATPases that move calcium ions out of sarcoplasm into the extracellular space and into the lumen of the SR, respectively. Sodium/calcium exchangers in the sarcolemma allow three sodium ions to enter the cell in exchange for a single calcium ion. They are coupled to a sarcolemmal sodium/potassium transporting ATPase which pumps sodium (that has entered the cell in exchange for calcium) out of the cell in exchange for potassium to maintain the transarcolemmal sodium gradient.

Myosin phosphorylation and smooth muscle contraction (Figure I-1)

Electromechanical coupling and pharmacomechanical coupling both result in a rise in the cytosolic $[Ca^{2+}]$. Intracellular free calcium ions bind calmodulin in a 4:1 ratio (27). This binding induces a conformational change in calmodulin and exposes active sites on calmodulin that in turn bind to and activate myosin light chain kinase (MLCK). Activated MLCK catalyzes phosphorylation of myosin at the S19 site in each of the two 20-kDa light chains, triggering cycling of myosin cross-bridges along the actin filament and hence smooth muscle contraction (51).

Relaxation of smooth muscle occurs after the cytosolic [Ca^{2+}] returns to the resting level via mechanisms described above (27). Dissociation of calcium and calmodulin from MLCK follows and the inactive form of MLCK is generated. Phosphorylation of myosin ceases, and the myosin that had been phosphorylated during the contractile state is dephosphorylated by myosin light chain phosphatase (MLCP) (52). Dephosphorylated myosin detaches from actin, and the smooth muscle relaxes.

Secondary calcium-dependent control mechanisms

There are secondary calcium-dependent mechanisms that can modulate actin-myosin interaction independent of myosin phosphorylation (53) and lead to smooth muscle contraction. It is possible that some of these mechanisms may play a role in vasospasm. Some of them use a thin filament regulatory mechanism involving the proteins caldesmon and calponin (actin/myosin is a thin/thick filament regulatory mechanism). Calponin and caldesmon are thin filament proteins that under resting conditions inhibit actin/myosin interaction and inhibit actomyosin ATPase activity (see below).

The "latch state". In the so-called "latch state" prolonged tension can be maintained in smooth muscle at a lower level of myosin phosphorylation. Dephosphorylation of an attached cross-bridge gives rise to a long-lasting, noncycling latch-bridge. Latch bridge formation is a form of energy conservation during prolonged tension generation because significantly less amounts of ATP are hydrolyzed during force maintenance than during force development. This maintenance of prolonged tonic contraction is achieved by noncycling or slowly cycling cross-bridges (54). However, latch bridges are calcium-dependent and relaxation in smooth muscle occurs when the cytosolic calcium concentration return to resting levels (55).

Caldesmon. In smooth muscle cells caldesmon interacts with calmodulin in a calcium-dependent manner and with actin in a calcium-independent manner (27,56). There are two forms of actin-containing domains in smooth muscle: the actomyosin domain and the actin-intermediate filament domain. Caldesmon is located in the actomyosin domain, stretching out along the actin double helix with tropomyosin.

Caldesmon binds to the actin filament rather than tropomyosin (57). The function of caldesmon is to inhibit the activity of actin-activated myosin magnesium-ATPase. Phosphorylation of caldesmon by calcium-calmodulin-dependent protein kinase II (CaM kinase II) in the presence of high [Ca^{2+}] results in the loss of its affinity for myosin and its inhibition of the actin-activated myosin magnesium-ATPase, thus allowing cross-bridge cycling to occur (58). Caldesmon can cross-link actin to myosin. This cross-linking may represent latch-bridge formation, and it may organize the contractile elements into a three-dimensional network capable of tension generation in the presence of appropriate stimuli (27).

Calponin. In smooth muscle cells calponin interacts with calmodulin in a calcium-dependent manner and with actin in a calcium-independent manner (59-62). It inhibits the actin-activated magnesium-ATPase activity of smooth muscle myosin through its interaction with actin; myosin phosphorylation is unaffected. Under physiological conditions calponin is phosphorylated by protein kinase C (PKC) while in the presence of elevated cytosolic [Ca^{2+}] it is phosphorylated by CaM kinase II. After phosphorylation calponin dissociates from actin and loses its ability to inhibit the actomyosin ATPase (59). The dissociation of calponin from actin allows maximal phosphorylation by MLCK and cross-bridge formation with maximal tension development. The level of calponin phosphorylation correlates with the velocity of muscle shortening; modulation is achieved by altering the relative amounts of phosphorylated calponin and myosin.

Other mechanisms in smooth muscle cell contraction. Calcium-independent and phospholipid-diacylglycerol dependent isoenzymes of sarcolemmal PKC also play role in the regulation of contraction and other cellular functions in smooth muscle (48,53,63). Activation of PKC in vascular smooth muscle modulates agonist-stimulated phospholipid turnover, increases the generation of contractile force, and regulates cell growth and proliferation (48). One mechanism probably involves the phosphorylation of delayed rectifier potassium channels by PKC and inactivation of MLCP. Another indirect mechanism may involve the phosphorylation of RAF (a serine/threonine kinase) by PKC and the activation of mitogen activated protein kinase (MAP kinase) which

phosphorylates caldesmon.

Tonic smooth muscle cell contraction. The actin-myosin and calmodulin systems regulate phasic smooth muscle cell contraction. However in a process such as cerebral vasospasm it is tonic smooth muscle cell contraction that is likely more important; how established mechanisms contribute to tonic contraction are under study (64-66). A coordinate fibrillar domain model of contraction has been used to try and explain the cellular and molecular events involved in the initial and sustained phases of smooth muscle contraction (67). This model differs from hypotheses such as the latch state. According to this model the initial phase is mediated by a rise in cytosolic calcium concentration with a resulting calmodulin-dependent activation of both myosin light chain kinase and dissociation of calponin and caldesmon from the actin-tropomyosin-myosin fibrillar domain. These events lead to an interaction between actin and the phosphorylated light chains of myosin. This initial phase is followed by a sustained phase in which a rise in intracellular calcium concentration stimulates the plasma membrane-associated, calcium-sensitive form of PKC that results in phosphorylation of both structural and regulatory components of the filamin-actin-desmin-fibrillar domain.

Role of endothelium in the responses of smooth muscle

Endothelial cells help to regulate the vascular tone of cerebral vessels (68,69) by producing relaxing factors such as endothelium-dependent relaxing factor (EDRF) (70), prostacyclin, endothelium-derived hyperpolarizing factor (EDHF), and oxygen-derived free radicals (68).

Nitric oxide. In extracranial vessels, EDRF has been identified as nitric oxide (NO) or a labile nitroso compound containing NO, such as S-nitrocysteine (71,72); in cerebral arterioles, EDRF may be an NO-containing compound rather than NO itself (73); in large cerebral arteries EDRF is probably NO since the pathway for NO production had been identified (74,75). In the following discussion EDRF and NO will be considered one and the same and referred to as NO. In the pathway for NO production, agonists such as bradykinin and substance P, act on the endothelial cell surface by binding to their receptors and, through a G-protein coupled mechanism,

produce IP_3; IP_3 then binds to its receptor on the sarcoplasmic reticulum leading to the release of stored calcium (76). Other agents, such as the calcium ionophore A_{23187}, promote direct entry of extracellular calcium. Intracellular free calcium, after binding calmodulin, interacts with nitric oxide synthase (NOS) which in turn leads to the conversion of L-arginine to NO. The NO diffuses out of the endothelial cell to nearby smooth muscle cells where it causes relaxation as described below (Figure I-3). Other agonists known to bind on the endothelial cell surface include acetylcholine, adenosine phosphates, thrombin, neurokinins, neuropeptide K, vasopressin, oxytocin, and histamine (77-79).

Isoforms of NOS. In cerebral arteries endothelial cells contain NOS while smooth muscle cells can be induced to express it (74,75). Two major isoforms of NOS are constitutive (cNOS) and inducible (iNOS) and they are regulated by different mechanisms (71,80). Inducible NOS, usually absent in macrophages and smooth muscle cells, has negligible basal activity but increases its activity significantly 2 to 4 hours following stimulation by lipopolysaccharide and/or gamma interferon (81) (ie. it is expressed after stimulation of the cell with cytokines). Inducible NOS acts in a calcium-independent manner. Constitutive NOS, present in neurons (nNOS) and endothelial cells (eNOS) acts in a calcium-dependent manner. After binding calmodulin, a complex is formed which acts as a cofactor for enzyme function (71,82). The formation of NO from L-arginine by NOS can be inhibited by NG-substituted analogues of L-arginine, such as NG-monomethyl-L-arginine (L-NMMA), NG-nitro-L-arginine (L-NNA), and NG-nitro-L-arginine methyl-ester (L-NAME) (71,82). These inhibitors can produce a dose-dependent constriction in cerebral blood vessels by lowering the basal level of cyclic guanosine monophosphate (cGMP) in smooth muscle cells even in the presence of an intact endothelium (see below).

Relaxation of smooth muscle. NO relaxes blood vessels by binding to iron in the heme at the active site of soluble guanylyl cyclase (GC) in smooth muscle cells, thereby activating the enzyme to generate cyclic guanosine monophosphate (cGMP) (71). cGMP may elicit muscle relaxation through influences on a sodium-calcium exchange system, by stimulating the phosphorylation of poorly defined substrates by cGMP-dependent

protein kinase, via direct action at a cGMP-coupled second messenger system, or as a consequence of cGMP-mediated activation or inhibition of phosphodiesterases (71,83). Specifically cGMP-dependent protein kinase phosphorylates a variety of substrates including Mg-dependent Ca^{2+}-ATPase (plasma membrane and sarcoplasmic reticulum membrane), the Na^{+}/Ca^{2+} exchanger (plasma membrane), the IP_3 receptor (sarcoplasmic reticulum membrane), phospholambin (sarcoplasmic reticulum membrane) and Ca^{2+} channels, as well as causing potassium channel hyperpolarization. Activation of these leads to a decrease in the intracellular $[Ca^{2+}]$ which leads to smooth muscle relaxation. Nitrovasodilators, such as sodium nitroprusside and nitroglycerin, which are endothelium-independent vasodilators, directly increase formation of cGMP and produce relaxation of cerebral vascular smooth muscle cells (84,85). Methylene blue inhibits soluble guanylate cyclase, reducing the relaxation of cerebral vessels in response to NO and nitroprusside (85-87).

Basal vascular tone. The basal release of NO from endothelial cells is important in counteracting the constrictor response of cerebral vessels exposed to vasoconstrictors such as norepinephrine, serotonin, and prostaglandins. Basal release of NO exerts more influence on the vascular tone of large cerebral arteries (88), whereas relaxing factors such as oxygen-derived free radicals (for example, the hydroxyl radical) may be more important than NO in the regulation of basal vascular tone in smaller cerebral vessels such as arterioles (68,89,90).

Impairment of endothelium-dependent relaxation. Endothelium-dependent relaxation is impaired in conditions that include vasospasm following aneurysmal SAH, cerebral ischemia, stroke, atherosclerosis, and chronic hypertension (68). The impairment is related to alterations in the release of NO and/or production of endothelium-derived vasoconstrictors such as endothelin. Following aneurysmal SAH the activity of soluble guanylate cyclase may be reduced, as shown in a canine model of SAH (87,91). Hemoglobin, in addition to its direct vasoconstrictor action, binds NO and destroys NO by the generation of superoxide anions, thus decreasing the availability of NO to smooth muscle cells (92). The breakdown of hemoglobin after SAH leads to the production of the superoxide anion radical (O_2^-) (see below). NO interacts with O_2^- to

produce the peroxynitrite radical ($ONOO^-$) which itself decomposes to form the hydroxyl radical (OH^-) which is highly toxic to smooth muscle cells. Endothelin levels in basilar arteries and cerebrospinal fluid (CSF) may be increased after SAH (93-96). Endothelin is produced by endothelial cells and is a potent and long-lasting polypeptide vasoconstrictor (97). Thrombin and oxyhemoglobin found in the blood and CSF after SAH have been shown to induce endothelin gene expression and increase endothelin production (97,98).

Pathogenesis of cerebral vasospasm

Spasmogens and pathways

The time course of angiographic cerebral vasospasm is fairly constant. Onset usually occurs at day 3 to 5 post subarachnoid hemorrhage, peaks at 7 to 10 days and resolves by 21 days (1). This knowledge has led investigators to search for a putative agent that becomes present coincidentally with the onset of vasospasm, and then disappears along a similar time line (11,92,99-101). However, the identification of a single, definitive substance has remained elusive.

Oxyhemoglobin. In a review article in 1991, Macdonald and Weir (92) stated that the current experimental and clinical evidence regarding vasospasm could be most satisfactorily interpreted by assuming that oxyhemoglobin is the cause of cerebral vasospasm that follows subarachnoid hemorrhage. After a subarachnoid hemorrhage erythrocytes are trapped in the blood surrounding cerebral arteries. It is known that aged erythrocytes and their degradation products are the ingredients of blood clots that produce vasospasm in animal models (92,102-104). Hemolysis leads to the release of oxyhemoglobin. Furthermore, it is known that hemoglobin compounds, particularly oxyhemoglobin, can contract isolated smooth muscle cells and arteries of many different animal species *in vitro* (105-108).

Free radicals. Oxyhemoglobin is involved in the formation of free radicals which may then participate in lipid peroxidation. There is evidence to support a role for free radicals in the development of vasospasm (109,110). In a series of clinical and experimental studies, Sasaki et al. (111,112) noted that the concentrations of lipid

peroxides in the cerebrospinal fluid and in the arterial wall were markedly increased after subarachnoid hemorrhage and that intracisternal injection of a lipid hydroperoxide, 15-hydroperoxyarachidonic acid (15-HPAA), mimics chronic vasospasm observed after SAH (112). This work, and that of others, has shown that the degree of vasospasm is correlated with the concentration of lipid peroxides in the cerebrospinal fluid in patients with SAH (111,113,114). Furthermore, there is a close temporal association of cerebral arterial narrowing with oxygen free radical formation and lipid peroxidation (115,116). Also, treatments aimed directly or indirectly at reducing the generation of lipid peroxides facilitate blood flow and reduce vasospasm after SAH (117,118).

During autooxidation of mammalian oxyhemoglobin to methemoglobin, superoxide anion radicals (O_2^-) are produced (119-121). In turn, superoxide radicals in the presence of water generate hydrogen peroxide, which reacts with the ferrous molecule (Fe^{2+}) in hemoglobin and releases hydroxyl free radicals (121,122). These free radicals catalyze lipid peroxidation (122), which in turn activates phospholipases, including phospholipase C, in the plasma membranes of smooth muscle cells (65,66,123). Phospholipase C triggers a chain of intracellular signals which lead to the elevation of the intracellular calcium concentration and ultimately smooth muscle contraction. Lipid peroxidation also triggers calcium influx from the extracellular to the intracellular compartment (65,66,123). It is believed that the initial rise in intracellular calcium is responsible for the phasic contraction of smooth muscle cells, but activation of the protein kinase C pathway may account for the tonic contraction of smooth muscle cells in chronic vasospasm (66,67,123-126).

Endothelins. The release of oxyhemoglobin from subarachnoid hemorrhage clot also leads to the release of endothelin-1 (an endothelium-derived vasoconstrictor), which has been shown to participate in maintaining the chronic state of narrowing seen in cerebral vasospasm (127-129). The endothelins actually comprise a family of compounds: endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3). These isopeptides differ in molecular structure and in function. The only isoform of endothelin produced by endothelial cells is ET-1 (128,129). Release of ET-1 from endothelial cells can be stimulated by oxyhemoglobin, thrombin, epinephrine, interleukin-1, arginine

vasopressin, angiotensin II, or transforming growth factor-β (129,130) (Figure I-4). Agents capable of stimulating the synthesis of ET-1 in cultured human vascular smooth muscle cells include angiotensin II, arginine vasopressin, transforming growth factor-β, platelet-derived growth factor AA, and epidermal growth factor (131). Most of these ET-1-inducing substances are present in the subarachnoid blood clot following aneurysm rupture.

The intracellular mechanism of ET-1 production is known and has been reviewed (97,130) (Figure I-4). Biosynthesis of endothelin is regulated at the messenger ribonucleic acid (mRNA) transcription level (128,132). The production of ET-1 results from the procession of a prepro-form of endothelin by endopeptidases to yield an intermediate peptide called big endothelin-1 (128,129). Big ET-1 is in turn transformed by a putative endothelin converting enzyme (ECE) to give ET-1 (97,130).

At least three cDNA clones of ET-receptor have been isolated: ET_A, ET_B, and ET_C (133,134). ET_A has a high affinity for ET-1 and ET-2, but not ET-3. ET_B has an equal affinity for all three endothelins. ET_C has a high affinity for ET-3 (97). The fact that endothelins have their own receptors is consistent with the observation that smooth muscle contraction induced by ET-1 is resistant to the following antagonists: α-adrenergic receptor, H1-histaminergic receptor, serotonergic receptor, cyclooxygenase, and lipooxygenase (128). The evidence for this is from early pharmacological studies and more recent molecular cloning studies. This suggests that endothelins indeed act on membrane receptors different from those of other vasoconstrictors on vascular smooth muscle cells. ET-1 may be produced by cerebral arterial endothelial cells (135). And furthermore, in addition to acting directly on specific receptors of smooth muscle cells to produce vessel wall contraction, ET-1 may also sensitize blood vessels to other constricting substances (136-138). This observation may at least partially explain prolonged and abnormal constriction in cerebral vasospasm.

Experimental evidence supports an *in vitro* and *in vivo* vasoconstrictor effect of ET-1 in cerebral arteries. Isolated canine basilar arteries contract in a dose-dependent manner in the presence of ET-1 (127,139) and intracisternal injection of ET-1 can induce sustained narrowing of canine basilar arteries for 24 to 72 hours (127). It is known that

ET-1 causes vasoconstriction of cerebral arteries only from the adventitial side as intraarterial administration of ET-1 has no appreciable effect on the caliber of the normal canine basilar artery (140). Extracellular calcium is required in order for ET-1 to cause vasoconstriction (138), and a calcium channel blocker, such as nicardipine, can inhibit its effect (128). Other experimental evidence suggests that ET-1 probably acts through a phospholipase C pathway leading to the breakdown of intramembrane phosphoinositides and subsequent intracellular calcium mobilization and protein kinase C activation to cause smooth muscle contraction (131,141-148).

Oxyhemoglobin can stimulate cultured endothelial cells to produce endothelin (149). There is evidence that ET-1 produced by endothelial cells in cerebral arteries acts in a paracrine fashion on the membrane receptors of adjacent smooth muscle cells, thus producing vessel wall contraction (135). In fact, cultured endothelial cells are known to secrete twice as much ET-1 towards adjacent smooth muscle than towards the luminal side (150). However, as compelling as a role for it may be in vasospasm, it is still uncertain just how important ET-1 is. This is partly because of conflicting evidence: some studies have demonstrated increased levels of ET-1 in the plasma and CSF of patients or animals with SAH (95,151-153), while others have not (154-156). And time course studies have not all been consistent: Yamaura and coworkers (96) showed that the immunoreactive ET-1 level in the basilar arterial wall was significantly elevated on day 2 of SAH but not significantly changed on day 7 of SAH when angiographic vasospasm was evident. Recently Pluta et al. (157) commented that endothelin-1 is released from astrocytes (but not endothelial cells) during hypoxia and that it is released from the brain after transient ischemia. They found that there was no relationship between ET-1 and vasospasm *in vivo* or between ET-1 and oxyhemoglobin *in vitro*. They concluded that the increase in ET-1 levels in the CSF after SAH from a ruptured intracranial aneurysm appear to be the result of cerebral ischemia rather than reflecting the cause of cerebral vasospasm.

Studies looking at endothelin converting enzyme (ECE) blockade have also produced conflicting results. Phosphoramidon, a nonspecific metalloprotease inhibitor, is known to inhibit the conversion of big ET-1 to ET-1 by ECE (156,158). Because the

vasoconstrictive potency of big ET-1 is considerably less than that of ET-1, the inhibition of ECE should ameliorate the biological effects of ET-1. Indeed, intracisternal administration of phosphoramidon has been shown to decrease cerebral arterial narrowing induced by intracisternal big ET-1 (159). In addition, intracisternal pretreatment of phosphoramidon prevented cerebral vasospasm following SAH in a canine model (152). However, other studies did not elicit any preventive effect of this inhibitor in the development of cerebral vasospasm after SAH (154,156,160).

Finally, the use of endothelin receptor blockers, has led to different results. The ET-1 receptor antagonists used in experimental models of SAH include synthetic peptides named BQ123, BQ485, FR139317, and Ro 46-2005 (154,161-163). BQ123 is a specific ET_A receptor antagonist that does not cross the blood brain barrier. It prevents the early cerebral arterial narrowing following SAH after intracisternal but not intravenous injection (161) and can prevent vasospasm after SAH in a monkey model (164). However, another study showed that daily intracisternal administration of BQ123 inhibited contraction induced by ET-1 but did not prevent experimentally induced cerebral vasospasm (155). Ro 46-2005, a synthetic orally active non-peptide antagonist of endothelin receptors, has been shown to reverse established contractions to ET-1 (161), while bosentan, a competitive antagonist of ET_A and ET_B, did not (164).

Adenosine triphosphate (ATP). Recent work by MacDonald et al. (165), initially in dogs, and then in primates indicates that ATP is a vasoactive compound found in high levels inside erythrocytes and that it may contribute to the vasospasm that occurs after SAH. In the primate study ATP placed into the basal cisterns of monkeys resulted in significant angiographic vasospasm 7 days later. The authors recommended further studies to see if ATP levels in the vicinity of cerebral arteries after SAH are sufficient to cause vasospasm.

Vasoconstriction theory of vasospasm

The vasoconstriction theory of vasospasm suggests that smooth muscle contraction is responsible for prolonged arterial constriction seen following subarachnoid hemorrhage (12,13,15,166,167). Of the two major theories it has garnered the most support over the

last few years. There is both morphological and biochemical evidence for this theory.

Morphological Evidence. From a morphological viewpoint, cerebral arteries pharmacologically vasoconstricted *in vitro* demonstrate medial thickening that is indistinguishable from the thickening observed in vasospastic arteries seven days after hemorrhage (13). Sustained vasoconstriction may produce alteration in the pharmacological and structural properties of smooth muscle cells. By irrigating exposed feline basilar arteries with either serotonin or calcium gluconate, Yoshioka et al. (168) found that vasoconstriction longer than ten hours was both pharmacologically irreversible and associated with myonecrotic changes seen with electron microscopy. Fujii and Fujitsu (105) found that incubation of cultured arterial smooth muscle cells with oxyhemoglobin for 24 hours resulted in irreversible progressive contraction of the cells and ultrastructural changes resembling myonecrosis. It seems possible, therefore, that prolonged constriction in response to oxyhemoglobin and/or its vasoactive byproducts such as lipid peroxides or prostaglandins induces a temporarily irreversible state associated with ultrastructural myonecrotic change.

Biochemical Evidence. From a biochemical viewpoint there is evidence of metabolic failure and trophic disturbances in cerebral arteries that occurs soon after a SAH. For example, adenosine phosphates rapidly diminish over the course of chronic vasospasm in canine basilar arteries (169). Smooth muscle cells in cerebral arteries constrict when exposed to pharmacological agents including serotonin, noradrenaline, prostaglandins, and oxyhemoglobin. These agents bind to membrane receptors and activate phospholipase C, which in turn leads to production of inositol trisphosphate (IP_3) and diacyl glycerol (DAG), which then initiate the biochemical cascade that leads to smooth muscle contraction. Interestingly, while smooth muscle contraction induced by potassium chloride via membrane depolarization is sensitive to calcium channel antagonists, the contractions induced by the compounds listed above are less sensitive (170). There is evidence that phospholipase C activation plays a role in the induction of vasospasm. In an experimental model, Vollrath et al. (66,123) noted that oxyhemoglobin produced a transient but highly significant rise in the intracellular concentration of inositol trisphosphate (IP_3), and that the sustained elevation in intracellular calcium level

was abolished by neomycin, a phospholipase C inhibitor.

Asano et al. (171) have show that cerebral arteries with established vasospasm are associated with a marked elevation of diacyl glycerol (DAG), which was not accountable by the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) alone; however, the source and mechanism for the increase in DAG has not been established. Diacyl glycerol is known to activate protein kinase C (PKC), which is important in the signal transduction of normal smooth muscle contraction (27,48). Activation of PKC shortly after SAH may mediate non-specific constriction to a number of agents during the early phase of vasospasm. Antagonists of protein kinase C (H-7 and staurosporine) could prevent vasospasm in beagles (172). Nishizawa et al. (125) measured PKC activity in the membranes and cytosol of vasospastic canine basilar arteries with (γ-^{32}P)-adenosine triphosphate. Because PKC is translocated to the membrane from the cytosol when it is activated, the translocation is an index of the activation. The authors found that membrane PKC activity in the SAH model was significantly increased compared to that in the control group. The percentage of membrane activity to the total was greater but the percentage of cytosol activity was less in the SAH vessels than in the control arteries. The results indicated that PKC in vascular smooth muscle was translocated from cytosol to membrane and was activated after SAH. They concluded that this was direct evidence for a key role for PKC in the development of vasospasm. As is often the case, there is some contradictory evidence regarding the role of PKC in vasospasm. For example, Takanashi et al. (173) found that the level of PKC was not elevated in the cultured cerebral smooth muscle cells exposed to hemolysate and Minami et al. (174) showed that the function of PKC (and calpain) in an experimental SAH model is not the same as that in vessels with pharmacologically induced tonic contraction.

Studies also suggest that other components in the intracellular pathway of normal smooth muscle contraction, such as calmodulin and calponin, are altered after SAH. Doi et al. have shown that the calcium binding proteins calmodulin and caldesmon, as well as calponin have altered function in vasospastic arteries (175). Another protein implicated in the maintenance of prolonged cerebral vasospasm is μ-calpain which may normally exist in a reciprocal relationship with its intrinsic inhibitor calpastatin.

Yamaura et al. (176) have shown that the activity of μ-calpain resulting from the balance of μ-calpain and calpastatin is enhanced continuously in vasospastic vessels (and that the level of calpastatin is reduced); this continuous activation of μ-calpain probably induces proteolytic changes of regulatory and structural proteins in vasospastic vessels (176). In the course of agonist-mediated vascular smooth muscle cell contraction, intracellular calcium and calmodulin mediate MLCK activation and subsequent phosphorylation of 20 kDa myosin light chain (MLC_{20}), which promotes contractile force generation through actin-myosin ATPase. However, using the rat femoral artery model of vasospasm (177), Harada et al. have shown that there is no significant difference between control and vasospastic arteries in the phosphorylation level of MLC_{20} (178), suggesting that a different mechanism, other than phosphorylation of MLC_{20} might be responsible for chronic vasospasm.

The role of altered calcium homeostasis has also undergone investigation in vasospastic vessels (27). Exposing cultured smooth muscle cells harvested from primate cerebral arteries to oxyhemoglobin induces a rapid and progressive increase of intracellular calcium over the next several days. Furthermore, exposure of canine basilar arteries to the calcium antagonist HA 1077 has been shown to attenuate vasospasm (HA 1077 inhibits PKC).

Structural theory of vasospasm

The impetus for the structural theory of vasospasm partly stems from the observation that despite the fact that most cases of vasospasm spontaneously resolve within three to four weeks of onset (1,11-13,92), there are times when cerebral vasospasm leads to persistent arterial stenosis (179). As originally suggested by Conway and McDonald (180) in 1972 it is likely that this is due to prominent intimal thickening, which may reflect a nonspecific pathological response related to the severity of the vessel wall injury. The structural theory of vasospasm, therefore, suggests that chronic vasospasm represents an injury response of the vessel independent of vasoconstriction, and involves necrosis, edema, leukocyte infiltration, cellular proliferation, and fibrosis within the vessel wall (17,18,20,21,181-183). The basic tenet of this theory is that,

though vasoconstriction may be responsible for early vasospasm, prolonged vasospasm occurs because smooth muscle cells retain a shortened configuration due to alterations of the extracellular collagen matrix of the vessel wall, a process mediated by myofibroblasts that proliferate in the cerebral arterial wall exposed to subarachnoid blood. In order to deemphasize the role of active vasoconstriction a number of alternate terms for vasospasm have been suggested (16,179,184-187) in order to better reflect a state of chronic structural narrowing.

There is both direct and indirect observational evidence that lends support to this theory. Until quite recently angiographic and clinical vasospasm had proven to be relatively resistant to pharmacological vasodilatation, deemphasizing the role of pathophysiologic vasoconstriction. Furthermore, certain morphological changes noted in vasospastic arteries tend to support a more permanent change in the vessel wall. For example, spastic vessels obtained from primates after experimental SAH have been shown to have relatively rigid walls compared to control animals (15,188), and markedly diminished *in vitro* contractile responses to agonists such as norepinephrine, serotonin, and potassium chloride (88,188-191). Clower et al. (16) have reported decreased vessel elasticity 6 days after SAH in a monkey model. Kim et al. (192) have shown that the rigidity of vessel walls was increased in the presence of total inhibition of myogenic tone, emphasizing the importance of non-contractile components of the vessel wall in SAH. Morphological changes have been described in human autopsy cases of cerebral vasospasm (179,180,182,183) and experimental SAH (16,179,193-196), the striking features being cellular proliferation and increased extracellular matrix in the subintimal layer and the tunica media (16,179,182,197), and myonecrosis. Biochemical analyses and ultrastructural studies have also been used to demonstrate an increase in the collagen matrix and the presence of fibrosis in vasospastic arteries (12,15,187,188,198).

Specially designed morphological modelling systems have been designed to study the pathogenesis and morphological changes associated with vasospasm. One such system, developed by Yamamoto and coworkers (17-21) is the myofibroblast populated collagen lattice (MfPCL), which is a modified fibroblast populated collagen lattice (FPCL) (20). The FPCL system is a three-dimensional *in vitro* model that simulates

wound healing, scar contracture, and fibrosis (199,200). It is created by mixing a cell suspension and collagen extraction together and allowing the mixture to polymerize, thus trapping the cells in the resulting lattice matrix. Yamamoto et al. (20) found that, upon suspension in a three-dimensional collagen matrix, the myofibroblasts obtained from cerebral arteries taken at autopsies from patients who died of cerebral vasospasm would extend filopodia into the matrix within 24 hours. The cells rearranged the structural network of collagen by pulling collagen binding sites along their surfaces, compressing the collagen matrix. Reorganization of collagen fibrils and MfPCL compaction occur in the absence of cellular shortening observed in the constriction of smooth muscle cells. Accordingly, the constriction in vasospastic vessels is considered the result of the action of myofibroblasts on collagen, and perhaps on other components of the extracellular matrix (20). Bloody CSF from patients with symptomatic vasospasm accelerated MfPCL contraction (20). These authors also found that, when stimulated by CSF from patients with symptomatic vasospasm, myofibroblasts could compact collagen lattice at a rate equivalent to that of human dermal fibroblasts (20). MfPCL compaction was inhibited by verapamil or heparin but not by nimodipine or nicardipine (21). This observation suggests that proliferative cells appearing in vasospastic arteries have characteristics different from those of differentiated smooth muscle cells in the medial layer and could generate a force rearranging the proliferated collagen matrix present after SAH. The results from the studies in MfPCL systems have limitations. The cell lines used to populate the lattice system were derived from the media of cerebral arteries taken at autopsy from a few patients with cerebral vasospasm. The definite identity of these cells is still debatable, although the authors noted in these cells a limited number of ultrastructural and immunohistochemical characteristics consistent with myofibroblasts. Moreover, no controls are provided from normal human cerebral arteries, tainting the authors' conclusion that myofibroblasts are unique to cerebral arteries in spasm. Furthermore, there is evidence that smooth muscle cells may alter their phenotypes to resemble myofibroblasts when in culture (36,201-203).

Support for the structural theory of vasospasm has waned somewhat over the last few years. This is because there is accumulating evidence to suggest that cerebral

vasospasm cannot be fully explained by the structural theory. Pathological studies reveal that fibrosis develops late after SAH, usually following the period of worst myonecrosis (12-15). In a primate model of SAH, Findlay and coworkers (12) noted that arterial narrowing and vessel wall thickening in vasospastic arteries were due primarily to medial constriction. Macdonald et al. (166), using amino acid analysis and immunofluorescence microscopy in the same model, did not find increases in arterial collagens after SAH except when intimal thickening developed 28 days after SAH. Nagasawa et al. (15) quantitatively measured the collagen content of dog basilar artery following SAH and found progressive increases that became significant 14 days after SAH. Furthermore, a study by Mayberg et al. (167) suggested that structural changes in cerebral arteries after SAH do not directly contribute to vessel narrowing through increases in wall mass. Although Smith et al. (18,182,187,198) and other groups reported qualitative increases of collagen in the tunica media of vasospastic arteries under light and electron microscopy, others failed to notice fibrosis in arterial walls of vasospastic arteries from patients with SAH and animal models of SAH (12,180,204-207). Moreover, since vasospasm usually resolves by two to three weeks following SAH, one would expect fibrosis to do the same if it were holding arteries in a narrowed state. The structural theory is further weakened by the observation that certain pharmacological agents such as high-dose nicardipine (208) and intra-arterially administered papaverine (209,210) can in fact reverse cerebral arterial narrowing in vasospasm.

Inflammatory and immunologic theories of vasospasm

There is some evidence that an inflammatory and/or immunologic process may be involved in the pathogenesis of cerebral vasospasm. Chyatte et al. (211) implicated an "inflammatory vasculopathy" as a causative factor for vasospasm after observing improvement in patients treated with methylprednisolone post subarachnoid hemorrhage. In a primate model of vasospasm, Bevan et al. (188) noted that inflammatory cells are often found in the subarachnoid space and in the walls of vasospastic vessels. Immunosuppressive agents such as cyclosporine have also been shown to reduce arterial narrowing in a canine model of vasospasm (212). However, despite these observations,

the inflammatory and immunologic theories of vasospasm have not gained much support, partly because there are common inflammatory conditions such as basal meningitis which are not associated with vasospasm.

Newer concepts on the pathophysiology of vasospasm: the tyrosine kinase pathway

In the last few years a new signalling pathway for G-protein coupled receptors and growth factor/cytokine receptors, called the tyrosine kinase (TK)-mediated signal transduction pathway, has come under study with respect to normal and pathological cellular processes (213-218) (Figure I-5, right side). The mechanism is described briefly. Agonists such as growth factors and cytokines bind to a receptor on the plasma membrane of the cell (in Figure I-5, a smooth muscle cell). Tyrosine phosphorylation of the growth factor receptor follows, catalyzed by intrinsic TK. Then there is activation of a small GTP-binding *ras*-protein (RAS) via the growth receptor binding protein (Grb_2) and a guanine nucleotide exchange factor (m-son of sevenless, mSOS) in conjunction with *src*-homology (SH_2 and SH_3) domains; this helps to replace GDP with GTP on *ras*. Next follows a sequential stimulation of cytoplasmic protein kinases known as the MAPK (mitogen activated protein kinase) signalling cascade, which links events at the cell membrane with changes in gene expression at the nucleus. Sequential components of this cascade are RAF (MAP kinase kinase kinase), MEK (MAP kinase kinase), and ERK (which is the same as MAPK). RAF is a serine/threonine kinase that is also activated by protein kinase C (PKC) and hence the cascade may be initiated by more conventional extracellular agonists such as those induced by hemoglobin. MAPK not only stimulates gene transcription via *jun/fos* (and hence cellular proliferation; smooth muscle cell proliferation in Figure I-5), it also phosphorylates caldesmon and phospholipase A_2 and thus can cause contraction in smooth muscle cells by this route.

Since it is known that the TK signalling pathway is present in smooth muscle cells (219,220), there is a possibility that it may be involved in cerebral vasospasm. The evidence for this includes: (1) free radicals are known to have a role in vasospasm (see above) and also stimulate TK, MAPK, DNA synthesis and smooth muscle growth; (2) some growth factors stimulate vasoconstriction with an associated increase in TK activity;

(3) some vasoconstrictors such as endothelin produce contraction in part by the TK pathway; and (4) smooth muscle cell proliferation, which may be a component of the underlying problem in vasospasm, may be partly controlled by the TK pathway. The role of the TK pathway in vasospasm is the subject of ongoing research at the University of Alberta.

Morphological changes in vasospastic arteries

Morphological changes in vasospastic arteries have been studied using animal models of subarachnoid hemorrhage (14,16,182,184,188,193,194,221) as well as human autopsy specimens (180,182,204,205,222). These studies have looked at both gross and ultrastructural characteristics of vasospastic vessels using techniques that include perfusion fixation, immersion fixation, light microscopy, scanning electron microscopy and transmission electron microscopy, as well as other sophisticated biochemical methods. A number of consistent alterations have been noted in both animal and human vasospastic vessels.

Animal studies. Research using animal SAH models (4,16,182,184,188,193,194,221,223) has consistently revealed early degenerative changes in the endothelial cell layer, including vacuolization, disruption of interendothelial tight junctions, and occasionally endothelial desquamation and luminal microthrombosis. The tunica intima, like the overlying internal elastic lamina, is frequently convoluted due to contraction of the media (12,13). In addition, there is some degree of intimal thickening variously ascribed to edema, polymorphonuclear leukocyte infiltration, granulation tissue, migration (presumably from the media) and proliferation of smooth muscle cells or fibroplasia and collagenization. Alksne et al. (184) have suggested that cellular proliferation and fibrosis in the intima after SAH may be similar to that seen early in atherogenesis, perhaps indicating a fundamental arterial wall response to various noxious stimuli. Some workers have speculated that endothelial injury, followed by leukocyte or platelet adherence and release of chemotactic and mitogenic factors, may be important in the intimal reaction observed after SAH (224,225). Ultrastructural changes in the endothelium demonstrated in animal models often precede any significant hyperplastic

intimal changes evident on light microscopy (12,13). In a study by Findlay et al. (12) in a primate model seven days after SAH, when arteriographic narrowing is maximal, transmission electron microscopy revealed that endothelial cells were swollen and vacuolized and sometimes lost tight junctions with neighbors. The subendothelium was slightly edematous and in places became hypercellular with myointimal cells, but the large increase in vessel wall thickness was due to medial contraction (12).

Using the same primate model as Findlay et al. (12), Macdonald et al. (13) showed that there is a similarity between vasospastic smooth muscle cells and smooth muscle cells from arteries contracted with $PGF_{2\alpha}$, suggesting that smooth muscle contraction occurs during vasospasm after SAH. Muscle cells of vasospastic arteries and of arteries exposed to oxyhemoglobin, as well as those from arteries contracted with $PGF_{2\alpha}$, showed convoluted and folded cell membranes (13). Dense bodies, which represent attachment sites of intracellular contractile filaments to the cell surface between the folds of membrane, have been observed (13,226). Fay and Delise (227), using isolated smooth muscle cells from *Bufo marinus*, correlated smooth muscle contraction with evagination of the cell membrane, changes in myofilament orientation, and a decrease in cellular volume. They suggested that the contractile apparatus of the smooth muscle cell extends between attachment sites on the cell membrane that are relatively closely spaced. Indeed, a number of studies (226,228,229) have shown that convolution or corrugation of smooth muscle membranes occurs during sustained muscle contraction. However, a significant difference exists between vasospastic arteries and those contracted pharmacologically: degenerative ultrastructural changes in the endothelium and media were seen only in SAH-induced, chronic vasospasm, and these changes remained at 14 days despite considerable resolution of arterial narrowing and medial thickening (12,13). Muscular degeneration such as this has been observed in the majority of animal models and human series (16,180,184,222). It is partly because of this observation of a more permanent change in the walls of vasospastic vessels, that the structural theory of vasospasm has maintained its support. It may also partly explain why vasospasm cannot be reversed by certain pharmacological agents.

A number of studies have been conducted looking at morphological changes in vasospastic arteries along an extended time-course or at particular time-points, and have noted consistent changes in the first two weeks post subarachnoid hemorrhage (SAH), with gradual resolution to a relatively normal appearance six to 24 months later (14,16,184,193-195,230-231). In 1974, Fein et al. (193) studied SAH-induced ultrastructural changes of cerebral arteries in rhesus monkeys over a time course of approximately one week. The ultrastructural characteristics of vessels were examined after allotting them into three groups: 0-1 day post SAH induction (early spasm), 2-7 days post SAH induction (prolonged spasm), and greater than 7 days post SAH induction (chronic spasm). The vessels in the early spasm group showed a reduction in the luminal size with corrugation of the internal elastic lamina (IEL) which on the light microscopic level was not distinguishable from normally constricted arteries. However, on the ultrastructural level, within 8 hours of SAH, there were lipid figures, as well as lysosomal and mitochondrial degeneration noted in some of the smooth muscle cells. Vessels in the prolonged spasm group showed more muscle cell degeneration with intracytoplasmic vacuoles, loss of tight junctions between endothelial cells with platelet-fibrin thrombus deposition on altered endothelial surfaces, and nerve fiber degeneration. Vessels in the chronic spasm group showed progressive intracellular degeneration in smooth muscle cells and increased extracellular collagen matrix.

In another time-course study performed in 1978, Tanabe et al. (195) followed the sequential ultrastructural changes in the cerebral arteries from a canine SAH model up to 24 months after SAH. Within two hours after SAH, they noted rounding of endothelial cells, thickening of subendothelial substance, and vacuolation in smooth muscle cells. Six hours after SAH, increased "smooth muscle-like cells" (today referred to as myointimal cells or myofibroblasts) were observed in the intima, as well as increased numbers of dense bodies and vacuoles in smooth muscle cells. Forty-eight hours after SAH, these changes, as well as intimal thickening, were even more prominent. Between 3 and 7 days post SAH, detachment of some endothelial cells from each other and from the IEL, as well as degeneration of more muscle cells was observed. At 1 month after SAH, the endothelium returned to normal but intimal thickening was

still apparent. The most striking finding at this time was the widespread increase in collagen matrix in the media. At 4 months, the intima nearly returned to normal except for sight subendothelial thickening, and the smooth muscle population in the media was significantly reduced. At 12 months, the intima was unchanged. Some remnants of intracellular lysosome-like dense bodies in many of the smooth muscle cells could still be noted in the media which was otherwise normal. At 24 months after SAH, the intima looked normal and the media would have appeared normal if not for the presence of occasional dense bodies in the smooth muscle cells and in the extracellular space.

More recently, in 1995, Macdonald et al. corroborated many of the findings from earlier studies, performing amino acid analyses in conjunction with light and electron microscopy on vasospastic rabbit arteries (231).

Many of the studies described above have noted that the tunica intima of vasospastic arteries is thickened. In the past, on occasion, this was attributed to either an inflammatory or hypertrophic reaction within the media. However, studies employing morphometric analyses (12,14) have demonstrated that the observed thickening in the tunica media after SAH was not associated with a proportional increase in the volume fraction of this layer. This supports the concept that thickening of the media after SAH probably reflects configurational alteration related to smooth muscle contraction and/or rearrangement within the extracellular matrix rather than an actual structural mass increase (13,166). The shortening and overlapping of smooth muscle cells in the constricted media could lead to convolution of the underlying intima and elastic lamina (12).

It has been noted that, following SAH, some myofibroblast cells tend to migrate into the subendothelial space that exists between the endothelium and internal elastic lamina and then proceed to proliferate (14,193,195). The precise role of these cells in vasospasm is still being investigated; even their origin is uncertain. As reflected in their name, some studies suggest that myofibroblasts originate from fibroblasts in the tunica media (18,19,182), while others studies suggest that they originate from smooth muscle cells in the tunica media (166,232,233).

Human autopsy studies. Using light and electron microscopy to study human postmortem cerebral artery specimens, it has been shown that swelling within the tunica intima and tunica media occur within a few days of aneurysmal SAH (180,182,205,222). After one to six weeks, there is increasing intimal proliferation and necrosis of smooth muscle cells in the tunica media with subsequent narrowing of the arterial lumen (14). From 3 to 15 months post-SAH, the media becomes fibrotic and luminal narrowing gradually reverses (14). Hughes and Schianchi (205) found that in those patients surviving 17 days or fewer from aneurysm rupture the tunica intima was only slightly swollen, and necrosis of the tunica media as well as irregularities of the tunica elastica were present; in those surviving longer, in addition to medial fibrosis, the intima became the most abnormal component of the arterial wall, showing concentric thickening with fibroblasts, collagen fibers and foamy macrophages. However, other human autopsy studies have found minimal arterial changes, at least during the first two weeks following SAH (204).

Pharmacological changes in vasospastic arteries

The reactivity of vasospastic and normal arteries in the presence of various vasoconstrictors and vasorelaxants has been examined in both experimental and clinical situations. Both intracranial and extracranial vessels have been studied. Some of the results are outlined below. In reviewing these studies it must be kept in mind that there are differences in the intrinsic properties of different arteries ie. between human and animal; between different animal species; between intracranial and extracranial vessels in the same species; even between different arteries within the circle of Willis itself (35,234).

Vasoconstrictor studies: general. Numerous vasoconstrictors have been used in animal and human studies involving normal and vasospastic arteries, including potassium chloride, noradrenaline, serotonin, prostaglandins, endothelins, thrombin and oxyhemoglobin (188-191,234-246). Potassium chloride causes smooth muscle contraction by acting on voltage-gated channels whereas all the other agonists probably act via receptor-operated mechanisms and the phospholipase C pathway. Both increased and

decreased responses of human and animal cerebral arteries to agents such as catecholamines and serotonin have been observed.

Vasoconstrictor studies: increased sensitivity. Lobato et al. (238) noted increased vasoconstrictor response to noradrenaline and serotonin in feline posterior communicating arteries after SAH. This heightened sensitivity was maximal 3 days after SAH and slowly decreased afterward. The contractile response to serotonin was still significantly different from that found in controls up to four weeks post SAH. A significant increase in the contractility of canine vessels three and seven days after SAH has been reported by one group (240), although in another time course study in rabbits, serotonin hypersensitivity did not parallel the development of the more protracted spasm seen in humans (245). Ultrastructural changes, such as disintegration and disappearance of vesicles in the adrenergic terminals (247,248) and an increase in the maximal binding of norepinephrine to adrenergic receptors (249,250), may be a reflection of the impaired and abnormal adventitial adrenergic nerve function seen in certain experiments (188,237,239,242,251). Loss of catecholamine fluorescence after SAH has been demonstrated in the basilar arteries of rats (252), rabbits (253-255), and cats (237,253). Partly based on these results, denervation hypersensitivity has been proposed by some as a mechanism to explain the intensified response of vasospastic cerebral vessels to catecholamines. However, several studies have shown that in the basilar artery of rabbits (253) and cats (256) it is not possible to identify increased sensitivity to histamine, serotonin, norepinephrine, or potassium after chemical or surgical sympathetic denervation, suggesting that sympathetic adrenergic nerves exert little or no tonic influence in normal cerebral blood vessels.

Vasoconstrictor studies: decreased sensitivity. The preponderance of experimental evidence indicates that vasospastic arteries show a diminished response in the presence of vasoconstrictive agents. In work performed in 1977, Toda et al. (191) found that canine blood vessels were mildly hyposensitive to serotonin at 1 and 7 days after experimentally-induced hemorrhage. Then, in 1979, Simeone et al. (241) showed a decreased sensitivity of bovine cerebral vessels to noradrenaline and serotonin after denervation with 6-hydroxydopamine. Also in 1979, Boisvert et al. (189) found that

injections of blood containing serotonin into the subarachnoid space of monkeys produced only a transient constrictive response. In 1983, Nagasawa et al. (190) reported that the contractile response of the spastic arterial wall to serotonin remained unchanged after SAH although spastic constriction increased progressively and became maximal seven days after SAH. The authors measured the incremental elastic modulus of the canine basilar arteries subjected to experimental SAH, and found that the elastic modulus was significantly lower in the SAH group compared to the control. The elastic modulus correlated well with the ratio of collagen to elastin contents in the post-SAH period. In 1986, using a monkey model of chronic cerebral vasospasm, Bevan et al. (188) showed a marked reduction in the capacity of the vessel wall to contract, and a reduction in constrictor and dilator nerve influences on vascular tone. This was attributed by the authors to increased rigidity of the vessel wall secondary to inflammatory infiltrate and fibrosis. In 1995, Macdonald et al. (246) found that vasospastic rabbit carotid arteries were less responsive to potassium chloride and serotonin 3, 7, and 30 days post SAH. Even more recently, Onoue et al. (257) measured the isometric tension generated in helical strips of human cerebral arteries obtained at autopsy 8 to 19 days post SAH upon exposure to noradrenaline, serotonin and prostaglandin $F_{2\alpha}$. In comparison to normal controls, the responses were reduced. A number of possible explanations have been put forth in order to explain the reduction in responsiveness observed in vasospastic arteries. These include endothelial degeneration, myonecrosis and phenotypic change, proliferative vasculopathy with increased myofibroblasts and extracellular collagen in vessel walls, and an inflammatory infiltration of vessel walls with decreased distensibility (183,185,195,196,258,259). It is also possible that vasospastic vessels are already severely constricted and thus cannot respond as dramatically as nonvasospastic vessels (236). More recently alteration of intracellular signalling of smooth muscle constriction has been increasingly recognized in vasospastic vessels (260); however, the exact intracellular signal transduction responsible for the decreased response of vasospastic vessels to vasoconstrictive agents is still undetermined.

Vasorelaxants: general. Vasorelaxants may be either endothelium-dependent or endothelium-independent, also known as direct smooth muscle relaxants. Endothelium-

dependent vasorelaxants include the calcium ionophore A_{23187}, bradykinin, acetylcholine, substance P, thrombin, adenosine diphosphate and serotonin, while direct smooth muscle relaxants include papaverine, PGI_2, atrial natriuretic factor (ANF), calcitonin gene related peptide (CGRP), sodium nitroprusside and nitroglycerin. Both kinds have been used in the study of vasodilatation in normal and vasospastic cerebral vessels (71,76-80,85-87). As in the case of vasoconstrictors different results (79,235,236,244,261,262) have been obtained, however, most studies have shown that vasorelaxation is impaired in vasospastic vessels.

Vasorelaxants: endothelium-dependent. Kim et al. (235) reported that the decrease of *in vitro* endothelium-dependent vasorelaxation was proportional to the degree of *in vivo* vasospasm in a canine SAH model. In the same experiment it was shown that the intraluminal release of NO was not impaired suggesting that the loss of endothelium-dependent relaxations during vasospasm was either due to an abnormal diffusion of NO or to a loss of responsiveness of the smooth muscle to the factor. Using a rabbit model of SAH, Vorkapic et al. (244) showed endothelium-dependent vasodilatation to acetylcholine was diminished. The highest degree of *in vivo* vasoconstriction was seen when endothelium-dependent vasorelaxation was impaired least. Therefore, loss of endothelial function might enhance the vasoconstrictor influences of substances released from the clot, such as serotonin or thrombin as suggested by the authors. Other studies have shown that endothelium-dependent relaxations in rabbit and dog cerebral arteries mediated via NO are abolished by treatment with hemolysate and oxyhemoglobin *in vitro* (263,264) or by subarachnoid injection of blood *in vivo* (235,261,265). Most recently, Macdonald et al. (246) have shown that vasospastic arteries are less responsive to acetylcholine 3, 7, and 30 days post SAH in a rabbit model. Also, studies using human cadaveric specimens (266), have shown that vasospastic vessels are less responsive to endothelium-dependent relaxation elicited by substance P and bradykinin (as well as being less responsive to the nonendothelium-dependent agents nitroglycerin and prostaglandin I_2).

Hemoglobin inhibits endothelium-derived relaxing factor or NO in cerebral as well as systemic arteries. Hemoglobin inhibits the increase in cyclic GMP caused by nitrovasodilators as well as NO (235,263,267). Hemoglobin captures nitric oxide (268), which is the active intermediate of nitrovasodilators and is the mediator of endothelium-dependent relaxation. During vasospasm after SAH, hemoglobin is likely to be present in the vicinity of the smooth muscle, since infiltration of blood components is observed in the adventitial layer (194) and disruption of interendothelial junctions also occurs (269).

Management of cerebral vasospasm: prophylaxis and treatment

The management of vasospasm has greatly improved over the past three decades (2,270) and some of the conventional as well as newer therapies will be outlined below. In general these patient are very ill and managed in an intensive care setting. It is important that cerebral vasospasm is correctly identified as the cause for the patient's clinical deterioration. The setting and time course of symptom onset are important clues. Generally symptoms of vasospasm start between 3 and 14 days post aneurysmal subarachnoid hemorrhage (1). There is a well recognized but imprecise correlation between the amount of subarachnoid blood detected on initial CT scan and the likelihood of developing vasospasm (271). Confirmation is made by transcranial Doppler ultrasound and cerebral angiography. The extent of cerebral narrowing usually, but not always, correlates with the severity of symptoms. Alternate or concurrent pathology also needs to be ruled out.

Improving cerebral blood flow to areas of ischemia

In 1951, Denny-Brown (272) was probably the first to note that improvement of neurological function following a brain ischemic episode is often associated with a rise in blood pressure. In 1967, Farhat and Schneider (273) observed that elevating the systemic blood pressure could improve the intracranial circulation in patients with cerebrovascular insufficiency, with immediate improvement in symptoms of hemiparesis. In 1972, Wise et al. (274) successfully used vasopressor drugs to treat patients with focal

brain ischemia. In 1974, Kosnik and Hunt (275) showed that elevation of the systemic arterial pressure in seven patients with intracranial arterial aneurysms was effective in alleviating ischemic symptoms attributed to cerebral vasospasm. Subsequently a number of larger series have shown that symptoms of cerebral ischemia secondary to insufficient cerebral blood flow from vasospasm could be reversed by volume expansion and/or elevation of systemic blood pressure (7,276-278). Improvement in cardiac output by augmentation of preload and increasing cardiac contractility (7,276,277), in addition to decreasing blood viscosity through hemodilution (276), have also led to better outcome in patients with vasospasm. All of these treatment modalities share one common feature, which is an increase in cerebral blood flow (CBF). Cerebral blood flow is dependent on cerebral perfusion pressure which, in turn, is directly proportional to systemic blood pressure. In addition, CBF is also affected by blood viscosity. The standard treatment for delayed ischemic neurological deficits (DIND) secondary to vasospasm is the so-called "triple-H therapy" (hypervolemia, hypertension and hemodilution) (7,273-278). In most circumstances, this involves the use of crystalloid and colloid solutions to increase circulating blood volume in the patient, lowering the hematocrit to 30 to 35 percent, and elevating systolic blood pressure to 180 to 240 mm Hg. If necessary, cardiac inotropes such as dopamine or norepinephrine are added to elevate blood pressure. This therapy is frequently only partially effective and sometimes ineffective in reversing neurological deficits secondary to vasospasm. Potential risks of this technique include aneurysmal rupture if the blood pressure is elevated too high in patients with unsecured aneurysms, and pulmonary edema and cardiac failure in patients with compromised cardiorespiratory status (7). For the latter reason these patients usually require assisted ventilation and monitoring in an intensive care unit. As an extension of triple-H therapy implemented after the onset of symptomatic vasospasm, patients with thick subarachnoid blood clot on admission computerized tomography (CT) scanning following aneurysmal SAH are usually maintained in euvolemia or slight hypervolemia (as well as normotension or slight hypertension) with albumin and crystalloid solutions. Fluid loading expands intravascular volume, which improves cerebral perfusion pressure and cerebral microcirculation (7,272-274,278).

Pharmacological treatment of vasospasm

Calcium channel antagonists. Since the increase in intracellular calcium concentration is a requirement for smooth muscle contraction in arteries, the idea of using calcium channel blockers was appealing. This led to studies involving drugs such as nicardipine (128,208) and nimodipine (279,280). Presently nimodipine, a dihydropyridine, is routinely administered to all patients admitted with SAH in an oral dose of 60 mg every four hours. Nimodipine is a lipophilic calcium channel blocker, and therefore crosses the BBB relatively easily. In 1988, the Canadian nimodipine trial (271) showed that nimodipine treatment was associated with a significantly better outcome (29.2% in nimodipine-treated patients compared to 9.8% of placebo-treated patients). Delayed ischemic deficits (DID) from vasospasm were significantly less frequent in the nimodipine group with permanent deficits occurring in 6.9% of nimodipine-treated patients and 26.8% in placebo-treated. However, angiography showed no significant difference in incidence of moderate or severe diffuse spasm which was seen in 64.6% of nimodipine-treated patients and 66.2% of placebo-treated patients. In 1989, the British aneurysm nimodipine trial group (281) reported the largest multicenter trial of nimodipine use in SAH patients. The authors did not find any significant reduction of angiographic vasospasm or mortality in the treatment group, although there was better outcome and decreased cerebral infarction. Thus, the evidence suggests that nimodipine improves outcome and reduces delayed neurological deterioration in patients with SAH by mechanisms other than prevention or reduction of vasospasm as visualized on angiography. Currently, it is still uncertain whether the salutary effect of nimodipine is on vessels or on neurons (protecting them against ischemia) (271), or both. It has been proposed that nimodipine improves collateral circulation to the ischemic areas (282).

Free radical scavengers and inhibitors of lipid peroxidation. The concept that oxygen free radical formation and iron-catalyzed lipid peroxidation are part of the cascade leading to arterial narrowing after SAH has led to the development of drugs designed to counteract this aspect of vasospasm development. Tirilazad mesylate or compound U74006F (a 21-amino steroid; also known as a lazaroid) is a potent scavenger

of free radicals and an inhibitor of lipid peroxidation (283,284). Since these processes are thought to be important in the final cascade of cell death (283), tirilazad has been studied in experimental models of stroke (283,285), head injury (283,286), as well as subarachnoid hemorrhage (283,287-291). In 1988, Hall and Travis (287) demonstrated that tirilazad mesylate retarded the development of post-SAH cerebral hypoperfusion in a feline model. In 1989, Steinke et al. used a primate model of SAH to demonstrate that tirilazad could ameliorate angiographic vasospasm and that it possessed a cytoprotective function. Tirilazad is well-tolerated and lacks glucocorticoid side effects in humans (292). This drug has undergone preliminary clinical testing. The Phase II Canadian Tirilazad Study (a part of the Cooperative Aneurysm Study) (292,293) compared placebo with tirilazad at doses of 0.6, 2.0, and 6.0 mg/kg/day for 10 days following SAH. All 245 patients enrolled received nimodipine. In the results reported in 1995 (293), there were no serious adverse medical complications due to the drug. A trend toward improvement in overall 3-month patient outcome was seen in the 2.0 mg/kg per day tirilazad group compared to the outcomes in the vehicle-treated groups. The authors concluded that tirilazad mesylate was safe in SAH patients at doses up to 6.0 mg/kg per day for up to 10 days and that it was a promising drug for the treatment of patients with aneurysmal SAH.

Vasorelaxants. If the vasoconstriction theory for the pathogenesis of cerebral vasospasm is most correct, then intuitively endothelium-dependent and endothelium-independent smooth muscle relaxants might have a role to play in the treatment of vasospasm. Indeed a number of different agents have been tried over the years with variable, but until recently mostly disappointing results. One of the main problems is that amelioration of vasospasm, if it occurs at all with the drug under study, is often only transient. In 1976, Allen and Gross (294), using a canine experimental model, reported some success in reversing pharmacologically induced arterial narrowing using six different agents: cyproheptadine, chlorpromazine, phenoxybenzamine, nitroprusside, papaverine, and phentolamine. In 1981, Gavras et al. (295) presented evidence in a canine model that angiotensin II might participate in cerebral vasospasm, and that therapy with an inhibitor of angiotensin-converting enzyme might be of some benefit. Also in

1981, Frazee et al. (296) showed that in a primate model of vasospasm, treatment with intravenous nitroglycerin resulted in a modest increase in the size of constricted vessels. Antithrombin III, another vasorelaxant, has also been shown to reduce vasospasm in a canine model (297).

To this date, the vasorelaxant with the most clinical promise has been papaverine (209,210,298-303). It is currently used to treat patients with cerebral vasospasm that has proven refractory to triple-H therapy and nimodipine. In back-to-back publications in 1992, Kaku et al. (209) and Kassel et al. (210) reported on the successful treatment of cerebral vasospasm in small series of patients using intra-arterially administered papaverine. Similar results were reported by Clouston et al. (298) in 1995. The use of papaverine has been associated with some problems: transient neurological events (300), thrombocytopenia (302), intracranial pressure changes (301), and crystal emboli (299). And though its therapeutic effects may be dramatic, they are often only transient.

Other pharmacological agents. A number of other pharmacological compounds to treat vasospasm have been investigated, mostly in experimental models. Success has been variable. These agents include: antibiotics, such as kanamycin (304) and neomycin (123); the neuroprotective agent, nizofenone (305); a selective antagonist of myosin light chain kinase, called ML-9 (306); a ribonucleic acid synthesis inhibitor (directed against endothelin), actinomycin D (307); a serine protease inhibitor, FUT-175 (308,309); other antioxidant and iron-chelating agents, such as deferoxamine (118) and U74389F (310); a free radical scavenging amino acid, histidine (311); intracarotid nitric oxide (312); copper-zinc superoxide dismutase (313); and the endothelin converting enzyme inhibitors mentioned earlier, BQ123, BQ485, FR139317, and Ro 46-2005 (154,161-164).

Mechanical and pharmacological clot removal

Since cerebral vasospasm arises as a result of the breakdown of blood clot products such as oxyhemoglobin in the subarachnoid space, intuitively early operation and removal of the blood clot by simple mechanical means during the acute stage of SAH might decrease the incidence of vasospasm and improve outcome. Experimental studies by Nosko et al. (314) and Handa et al. (315), and clinical studies by Suzuki et al. (98)

and other groups (316,317) support this presumption. Despite these encouraging studies, and though studies have shown that the timing of aneurysm surgery and surgical manipulation of arteries does not affect the development of vasospasm (318,319), total clot removal from the basal cisterns is technically difficult and poses a real danger to patients. For these reasons, mechanical clot removal is not a common practice.

To avoid difficulties associated with mechanical clot removal, fibrinolytic agents such as recombinant tissue plasminogen activator (rt-PA) (320-324), urokinase (325,326) and streptokinase (327) have been utilized to lyse subarachnoid blood clot. These agents are usually administered into the basal cisterns following surgical repair of the aneurysm. Preliminary data in humans (321,328-330) and animal models (320,322-324,327,331) have suggested favorable outcome from utilization of fibrinolytic therapy. Recently, Findlay et al. (332) reported on the results of a multicenter, randomized, blinded, placebo-controlled trial to study the possible role of intracisternally administered rt-PA in preventing cerebral vasospasm after SAH. The rt-PA group showed a trend towards lesser degrees of angiographic vasospasm. When only those patients with thick subarachnoid clots were considered, there was a 56% relative risk reduction of severe vasospasm in the rt-PA group, which was significant. The authors recommended continued study in a larger randomized trial.

Newer concepts in the treatment of vasospasm: gene therapy

Gene therapy is the introduction of genetic material into cells to provide temporary or permanent instructions for the prevention or treatment of disease (333). After several years of unfettered excitement, most workers in the field agree that gene therapy is at an early stage of development (334). Nevertheless, despite the obstacles, it holds considerable promise, especially for diseases involving the intracranial blood vessels (such as vasospasm) which are difficult to treat surgically and are often refractory to nonsurgical management (334).

Gene therapy for cerebral vasospasm might involve (333-335): (1) administration of a gene (into the CSF or blood) which encodes a vasoactive protein that inhibits vasospasm (after an aneurysm has been clipped); (2) administration of a gene that

encodes a protein that inhibits a proven spasmogen (eg. endothelin); and (3) administration of a gene which encodes a protein that stimulates the growth of collateral blood vessels into the area of brain deprived of blood (eg. vascular endothelial growth factor, VEGF). Problems with the strategies described above include (334,336): (1) difficulty finding suitable vectors; (2) difficulty targeting the appropriate tissues and cells; (3) difficulty delivering the vectors; and (4) difficulty predicting whether and where the gene product will function.

Another strategy that holds promise in the treatment of intracranial disease is the use of antisense oligonucleotides (337-339). Antisense oligonucleotides offer the potential to block the expression of specific genes within cells. Inhibition of gene expression by antisense oligonucleotides relies on the ability of an antisense oligonucleotide to bind a complementary mRNA sequence and prevent translation of the mRNA (337). This technique has been used with some success to help understand the pathogenesis of vasospasm (340,341) and has potential in its treatment and prevention. Using a rat basilar artery model of vasospasm, Onoda et al. (340) showed that an intracisternally administered preproendothelin-1 mRNA antisense oligoDNA could inhibit vascular contraction, indicating that ET-1 may play a role in hemolysate-induced vasoconstriction in rats. Using a rat femoral artery model of vasospasm, Onoda et al. (341) showed an inhibitory effect of antisense oligonucleotides on collagen induction and vessel contraction, indicating that the induction of procollagen type I may be involved in the pathogenesis of the arterial contraction induced by blood. Gene therapy for cerebral vasospasm is presently under study at the University of Alberta.

Transluminal balloon angioplasty

Definition and clinical perspectives

Definition. Transluminal balloon angioplasty (TBA) is an interventional technique whereby an inflatable balloon attached to the tip of a catheter is used to dilate arteries possessing narrowed lumens due various pathological processes. It has recently been applied to the treatment of cerebral vasospasm.

Development of TBA. TBA was first developed in 1964 when Dotter and Judkins (342) used a non surgical percutaneous transluminal approach for the treatment of atherosclerotic obstruction in peripheral vessels. Clinical application of their coaxial system and the addition of latex and caged dilating balloon catheters by Dotter et al. (343) and Porstmann (344) led to the development of double-lumen balloon catheters by Gruntzig and Hopff in 1974. Subsequently, in 1979, Gruntzig et al. (345) reported the use of a modified inflatable balloon catheter in performing percutaneous transluminal coronary angioplasty (PTCA; PTA) to treat atherosclerotic coronary artery disease. This coincided with the application of the technique to the treatment of atherosclerotic stenoses throughout the body; the abdominal aorta, as well as the brachial, axillary, common carotid, vertebral, subclavian, celiac, superior mesenteric, and hypogastric arteries were all successfully treated with PTA (346). Then in 1980, Saddekni et al. (346) and Fallon (347) reported on the use of percutaneous transluminal angioplasty of nonatherosclerotic lesions, such as fibromuscular dysplasia of the cervical carotid artery, congenital coarctation of the aorta and renal artery stenosis secondary to fibromuscular dysplasia or Takayasu arteritis.

TBA of cerebral arteries. Some 20 years after its first description, in 1984, Zubkov et al. (22) reported the first application of TBA to the dilatation of vasospastic cerebral arteries following aneurysmal SAH. A latex balloon, like that used in coronary angioplasty, was used. Reporting on 105 dilatations in 33 patients, they noted a generally favorable result with regression of focal neurological signs (22). In 1989 Barnwell et al. (348) described a silicone elastomer balloon specifically designed to treat cerebral vasospasm and were the first to report on its use in North America. Then later in 1989, in back-to-back publications Higashida et al. (23) and Newell et al. (24) reported the first large North American experience in the treatment of cerebral vasospasm using TBA. Both groups used specially developed silicone balloons attached to catheters; the former group reported clinical improvement in nine of 13 cases treated, while the later group reported improvement in eight out of 10 cases treated. Since then, a number of other clinical series have described the clinical use of TBA in cerebral vasospasm. Most reports have emphasized that TBA is effective in reversing angiographic and

clinical vasospasm, as well as increasing cerebral blood flow to the ischemic areas. The permanent nature of vasospasm reversal by TBA has often been inferred from sustained neurological recoveries, and in some series an unspecified number of patients were found to have no recurrence of arterial narrowing on follow-up angiography (23,24,230,349-355). In a description of 95 consecutive patients, all of whom underwent control angiography 5 to 7 days following angioplasty, Zubkov et al. (356) found no instances of progression of vasospasm in any dilated segment.

Present clinical use of TBA in cerebral vasospasm. TBA for cerebral vasospasm has gained increased usage primarily because it is relatively safe in experienced hands, it is often effective in cases of cerebral vasospasm refractory to maximal medical therapy, it works quickly, and its angiographic and clinical effects seem long lasting. The technique is successful in approximately 75% of selected patients (22-24,230,352,353,355-360). However, both the method and indications for cerebral TBA continue to be refined. At present TBA is generally reserved for cases of moderate to severe symptomatic vasospasm, in the absence of cerebral infarction, but following aneurysm repair, that have been refractory to conventional treatment measures such as hypervolemic and hypertensive therapy. However, it is not clear that these are the ideal conditions under which TBA should be employed and it may be that earlier implementation may optimize patient outcomes. In a retrospective study, Coyne et al. (361) concluded that the best results of angioplasty occurred when it was performed within a short time of symptom onset. Little benefit was seen in patients of poor clinical grade or when a new neurologic deficit had become established. They suggested that angioplasty be considered if hypervolemic, hypertensive therapy did not reverse the symptoms of vasospasm within 6 to 12 hours (361).

Complications associated with cerebral TBA. Although relatively safe in experienced hands, TBA is not without risk (23,24,362).

There is a danger of reperfusion hemorrhage (23,24). This is a hemorrhagic infarction in the vasospastic vascular territory, which can lead to death, and is thought to result from sudden increased blood flow to the infarcted brain tissue. Therefore computerized tomography (CT) or magnetic resonance imaging (MRI) should generally

be obtained prior to performing TBA to rule out a non-hemorrhagic cerebral infarction that may be converted to its hemorrhagic counterpart by the procedure.

A second potential risk of TBA is vessel rupture during balloon dilatation (362). Fortunately, this is relatively rare because the balloon catheter usually used for cerebral angioplasty is made of silicone elastomer which is gentle and gradual in expansion, therefore avoiding sudden distension and a sudden increase in wall tension which can precipitate vessel rupture. The small size of the cerebral angioplasty catheter (3.5 x 12.5 mm inflated) confers further protection against vessel rupture. The pressure exerted by this type of balloon is usually in the range of 50.7-152.0 kPa (0.5-1.5 atmospheres) (363), which is relatively low compared to 506.5-810.4 kPa (5-8 atmospheres) exerted by the polyethylene or latex balloons used for coronary angioplasty of atherosclerotic plaques (363). The silicone balloon also elongates and conforms to the luminal shape of the artery.

A third potential risk of TBA is re-rupture of an unclipped or incompletely secured aneurysm distal to the dilated segment (24,362). The untreated or partially treated aneurysm may be partially protected from hemodynamic forces and rebleeding by vasospasm in the proximal parent artery. Dilating this vessel might augment flow into the aneurysm and precipitate rerupture. Linskey et al. (362) reported a case of fatal rupture of the intracranial internal carotid artery during transluminal angioplasty for vasospasm; the authors concluded that the rupture probably occurred at the unclipped residual aneurysmal neck where wall structure was the weakest.

A fourth potential risk of TBA is occlusion of an arterial branch (24). Newell et al. reported a delayed infarction in the left middle cerebral artery distribution six weeks following an angioplasty procedure. An angiogram showed branch occlusion of the middle cerebral artery with no source of emboli revealed on workup.

Technical advances in cerebral TBA. The most easily accessible cerebral arteries for transluminal balloon angioplasty are the internal carotid artery, basilar artery and the M1 portion of the middle cerebral artery. Several reports have described refinement of cerebral balloon catheters which improve the steerability of the catheter, and modification of techniques to improve accessibility to more distal and selective branches such as the

A1, A2 and M2 segments (364-366). For instance, some of the modifications of basic techniques are "wire-in-balloon" and "wire-through-leak balloon" procedures as described by Brothers and Holgate (364). The "wire-in-balloon" method requires no alteration of the basic balloon catheter/wire system, but rather a change in the way it is used. When a distally curved wire is advanced into a deflated balloon, the balloon and wire tip will form a curve because of the elastic pull of the balloon. The radius of this curve is changed as more wire is advanced. The balloon can be steered by rotating the wire, and it can tolerate the internal pressure from the wire. The result is a steerable balloon tip with variable stiffness and curvature. For the "wire-through-leak balloon" technique, a hole is made in the center of the distal end of a contrast-filled balloon and a distally curved guidewire is inserted protruding through the hole. When the catheter does not advance easily, the balloon is inflated where it lies at the origin of a branch, or at the proximal end of a luminal narrowing, to dilate that arterial segment. This slight dilatation may facilitate subsequent engagement and entry of the narrowed segment by the balloon catheter. Terada et al. described a new microballoon, which is 2.2 x 6.5 mm in diameter when inflated with 0.02 mL of fluid, for use in the M2, A1 and A2 portions of cerebral arteries (366). This balloon is much smaller than the conventionally used cerebral balloon (3.5 x 12.5 mm diam. when inflated with 0.1 mL fluid).

Morphological and pharmacological changes in noncerebral vessels dilated with TBA

An understanding of the mechanism of action of TBA in cerebral vessels has only begun to emerge over the last few years. However, the pharmacological and morphological effects of TBA in extracerebral vessels has been established over the past two decades. These effects have been studied by researchers investigating the mechanism of action of coronary TBA. It is emerging that some the principles may apply to angioplasty of cerebral arteries.

Pathology of atherosclerosis. In this disease process, luminal narrowing is due to both circumferential and focal plaque formation. The morphology of the atherosclerotic plaque is known (367-369). Plaques consist of cholesterol crystals and a large amount of collagen, while their luminal surface is covered with endothelium.

The number of intimal smooth muscle cells is increased and many contain lipid droplets. The internal elastic lamina is irregularly stretched and in some areas absent. The tunica media is composed of increased intercellular ground substance and collagen, particularly in the inner layers, while the elastic fibers are irregularly arranged compared to the nonatherosclerotic vessels. The medial smooth muscle cells contain lipid droplets and/or degenerative changes, including swollen mitochondria.

Morphology of angioplastied coronary arteries. Autopsy specimens of human atherosclerotic coronary vessels treated with angioplasty (370-375), and specimens of coronary vessels angioplastied in animals (370,375-377), have been analyzed and compared. Findings include deposition of fibrin and platelets over denuded endothelium, tearing or cracking of atheromatous intima, dehiscence of the plaque and intima from the underlying media, stretching and tearing of the media, and stretching of the adventitia (370,373,375-378). It is the disruption of the atheromatous plaque and the stretching of the media and adventitia that account for the dilatation of the vessel lumen, and the potential for an intimal flap (373). In an editorial in *The Lancet,* coronary angioplasty has been referred to as a "controlled injury" to the vessel wall (378). Overdistension of the wall can cause dissection in the media and adventitia and possible vessel rupture (378). In a rabbit model, Zollikofer et al. (369) studied the correlation between histological changes in the vessel wall, balloon size, and balloon inflation time in both normal and atherosclerotic rabbit aortas after angioplasty. They noted that disruptive changes in normal aorta correlated in a linear fashion with duration of inflation and size of the balloon. However, in atherosclerotic aortas there was no such relationship. The authors attributed the poor correlation in the atherosclerotic vessels to the variation in distribution and thickness of the plaques. The underlying media of the thick plaque remained unaffected, but the vessel wall between the plaques became stretched in atherosclerotic aortas after angioplasty. Unlike atherosclerotic vessels, normal vessels which underwent balloon angioplasty showed uniform stretching of the entire wall.

Morphology of angioplastied vessels in nonatherosclerotic disease. Narrowing due to fibromuscular dysplasia dilates easily with TBA, which suggests a concentric stretching and shearing of the fibrous tissue, which then heals in its dilated state

(346,347). Intimal fibroplasia may be eccentric, and concentric stretching during dilatation may not be possible, leading to less satisfactory results. On the other hand, radiation-induced arterial stenosis involves periarterial fibrosis and arterial wall thickening, and TBA is less effective in these cases (379).

Pharmacology of angioplastied noncerebral vessels. Pharmacological studies have been performed on atherosclerotic and nonatherosclerotic coronary and peripheral arteries. Using dogs and rabbits, Castaneda-Zuniga et al. (380) showed that *in vivo* TBA caused immediate and permanent damage to the muscular component of the media. In this study Gruntzig balloon catheters were used to dilate normal canine carotid arteries and aortas of rabbits that had been fed a diet containing 2% cholesterol for 8 weeks. Arteries were examined immediately after and 2 months after TBA using pharmacological and structural techniques. Immediate damage to the muscular component of the media was demonstrated by lack of response to the vasoconstrictor vasopressin. Electron microscopy revealed extensive fragmentation of the muscle cells immediately following dilatation. The pharmacological impairment seemed to be permanent, as evidenced by the lack of constriction in these vessels 2 months after dilatation. The damaged smooth muscle cells had been removed by macrophages and replaced by collagen. It was concluded that paralysis of the muscular layer of the media is part of the mechanism of balloon dilatation.

An experiment by Wolf et al. (381) also showed that vessels dilated by *in vitro* balloon angioplasty decrease their response to vasoconstrictors. Circumferential strips of rabbit aorta were subjected to 60-second graded stretches *in vitro*. Before and after each stretch, the vasoconstrictor dose-responses to potassium and norepinephrine were measured. For stretch between 130 to 190% of resting length, vasoconstrictor responses were progressively enhanced. However, arteries stretched 190 to 220% of resting length showed a significant fall in developed tension in response to either vasoconstrictor. It was concluded that since arteries *in vivo* operate at 150 to 180% of resting length due to physiologic distension, it is likely that effective angioplasty induces paralysis of vascular smooth muscle. In an *in vivo* study using balloon angioplasty in normal rabbit aortas

(382), this same group noted that vasoconstrictor response was attenuated in proportion to the effective stretch applied to the aorta. When stretched beyond 15% of the original diameters, all arteries showed significantly decreased or abolished response to vasoconstrictor challenge. A study by Schweitzer et al. (383) studying pharmacological responses in rat aorta corroborated the findings of Wolf: that spontaneous and pharmacologically-induced vasoconstriction, whether receptor-mediated or voltage-mediated, is attenuated by balloon angioplasty. However, as is often the case, other studies have shown that blood vessels stretched *in vitro* increase their response to vasoconstrictors (384,385). The discordant results may be due to the different amounts of stretch applied in these studies.

In two separate experiments, Consigny et al. (386,387) performed *in vitro* angioplasty on nonatherosclerotic rabbit external iliac arteries with a balloon catheter in order to characterize and compare the immediate and long term effects, as well as the effects of short and long angioplasty times, on arterial contractions and passive mechanics. In the more recent study (387), contralateral vessels were dilated for either 20 seconds or 2 minutes, resulting in a 27-30% increase in their luminal diameters. Between the two sides, the vessels showed no difference in luminal diameters when measured angiographically prior to and following TBA. However, vasoconstriction with 70 mM KCl was reduced in the group dilated for 20 seconds, and this constriction was diminished even further with 2 minutes of dilatation. Both short and long dilatations resulted in similar biomechanical changes in the vessel wall, namely decreased wall thickness, increased elastic modulus, and increased circumferential wall stress (386). The authors concluded that inflating the vessels for 2 minutes offers no mechanical advantage but produces more smooth muscle cell dysfunction, which may reduce vasospasm and restenosis after angioplasty.

A number of experiments have examined the longer term effects of angioplasty and have attempted to determine why re-stenosis of the angioplastied vessels can occur (4,388-391). Two of the more recent studies are summarized below.

In 1990, Weidinger et al. (390) investigated the vasodilator function of endothelium that regenerated after balloon angioplasty and the relation of this function

to the extent of vascular injury and to subsequent intimal proliferation. Balloon angioplasty was performed on the left iliac artery of 47 New Zealand White rabbits. Vascular responses were examined *in vitro* 2 and 4 weeks after a "severe" injury (3.0 mm balloon) or a "moderate" injury (2.5 mm balloon). Both degrees of balloon injury caused complete endothelial denudation. Endothelium regrowth 2 weeks after either injury was confirmed histologically and by immunocytochemical staining. The regenerated cells had irregular sizes and polygonal shapes and lacked the typical alignment in the direction of blood flow. Endothelium-dependent relaxation of balloon-injured vessels to acetylcholine and the calcium ionophore A_{231287} were reduced at 2 and 4 weeks after severe injury. After moderate injury, endothelium-dependent relaxations to these agents were reduced at 2 weeks but had normalized by 4 weeks. Endothelium-independent relaxation to sodium nitroprusside, however, was preserved in all study groups. Morphometric analysis revealed an inverse correlation between the degree of intimal thickening and maximal relaxation to acetylcholine. Thus, there was a persistent attenuation of receptor-mediated endothelium-dependent relaxations after arterial injury. Although the regenerated cells had an altered morphological appearance, staining for factor VIII-related antigen confirmed their endothelial origin. The degree and duration of endothelial dysfunction depended on the severity of the initial injury, the time lapse since the injury, and was related to the extent of intimal thickness.

In the above study, because intimal proliferation was greater after severe than after moderate injury, it was suggested that the thickened intima formed a physical or a functional barrier to the actions of NO (390). NO has a short half-life that can be reduced further by superoxide anion (392), a product of macrophages (393), that may be present at sites of vascular injury (367). Smooth muscle proliferation progressed despite regeneration of the endothelium, and there was a significant correlation between degree of smooth muscle proliferation and loss of endothelium-dependent vasodilation (390). These observations indicate that the endothelium regenerating after balloon angioplasty may be dysfunctional with respect to its inhibition of vascular tone and regrowth. Since nitrovasodilators are known to inhibit proliferation of vascular smooth muscle *in vitro*, reduced release of NO from the aberrant regenerating endothelium could contribute to

intimal and medial smooth muscle proliferation after endothelial injury (390).

In 1991, in order to determine the time course of the return of endothelium-dependent relaxations and contractions during intimal regeneration, Cartier et al. (389) performed balloon endothelial denudation of thoracic and abdominal aortas of male Lewis rats and examined smooth muscle function and endothelium-dependent responses *in vitro* at 1, 2, 4, and 8 weeks after aorta injury. At each study interval during endothelial cell regeneration, vascular smooth muscle contracted and relaxed normally to direct stimulation with norepinephrine and sodium nitroprusside. Endothelium-dependent contractions to serotonin returned to normal at 1 week and developed into a hypercontractile response at 8 weeks. Endothelium-dependent relaxations to acetylcholine returned to normal at 8 weeks, but endothelium-dependent relaxations to adenosine diphosphate remained impaired. These experiments demonstrated that regenerating endothelium regained the ability to produce contracting factors before relaxing factors, and that it even exhibited potentiated contractile activity 8 weeks after injury. Thus, after direct arterial injury, regenerating endothelium has an abnormal endothelium-dependent function that could predispose the vessel to vasospasm and thrombosis.

The applicability of the findings in the above studies (389,390) performed in noncerebral vessels to cerebral vessels is uncertain since there is a preponderance of clinical and experimental evidence that cerebral vessels do not re-constrict after balloon angioplasty. An explanation for why a vasoconstrictor response may predominate over a vasorelaxant response under certain conditions of endothelial injury and regeneration has been offered (389). It is known that the vascular endothelium releases NO. NO relaxes vascular smooth muscle and inhibits platelet aggregation (394) and adhesion in normal blood vessels (395). However, vascular endothelium can also produce contracting factors such as superoxide anion (396) and endothelin (128), which mediate vasoconstriction. Superoxide anion can cause direct constriction of vascular smooth muscle (396) and has been identified as the possible mediator of endothelium-dependent contractions to arachidonic acid in the canine cerebral artery (118). Oxygen-derived free radicals also inactivate NO (397). Thus, the cumulative effect of superoxide anion would be scavenging of NO and direct contraction of the vascular smooth muscle. In conditions

that cause endothelial cell injury or regeneration, there is selective impairment in the release of NO, while the production of contractile factor is maintained or enhanced (389). This is the case after acute coronary occlusion and reperfusion (398), certain cases of cerebral vasospasm (87,235), atherosclerosis (399,400), and intimal regeneration after mechanical trauma (269).

Morphological and pharmacological changes in cerebral vessels dilated with TBA

The number of studies reporting morphological and pharmacological changes in cerebral vessels dilated with TBA has steadily increased over the past few years (22,181,231,246,357,363,401-407). Some of these are reviewed below.

In an abstract published in 1987, Pile-Spellman et al. (404) first reported on the acute physiological and anatomic changes in angioplastied arteries. In initial experiments, isolated canine cerebral arteries were angioplastied *in vitro* using a latex balloon (inflation diameter 8.5 x 4.5 mm) to maximum pressure of 1-3 atmospheres, giving +150-200% dilation for 20-40 seconds. Postangioplastied vessels did not constrict when exposed to 50 mM KCl. Histology showed disruption of the intima, media and adventitia. In a second series of experiments, canine basilar arteries were dilated *in vitro* using a latex balloon (inflation diameter 5 x 2.5 mm). Vessels that were gently angioplastied to 100-104% of native size showed mild endothelial dysfunction as evidenced by decreased substance P relaxation. Repeated or longer gentle angioplasty caused marked endothelial dysfunction with preserved muscle constriction upon exposure to KCl. Vessels preconstricted (-33%) with KCl (20 mM) and angioplastied back to native size, failed to constrict to KCl. Mild histological changes in these experiments included: areas of endothelial desquamation, occasional fracturing and dehiscence of the internal elastic membrane (IEM), with thinning of the muscle layer and one area of partial media fracture. The IEM of the perforating vessel origins was uniformly spared. Gross vessel integrity, was preserved. The authors concluded that angioplastying vessels to > +150% native size disrupts vessel layers; angioplastying preconstricted vessels back to native size causes vessel paralysis and mild histological damage; and gentle balloon manipulation such as is used in intracranial navigation can cause endothelial dysfunction.

In 1990, Chavez et al. (401) performed *in vivo* TBA in normal canine basilar arteries. They dilated the vessels with a silicone balloon to 130% of original diameter at 1.5 atmospheric pressure. On examination of the angioplastied vessels, they found areas of endothelial denudation with deposition of fibrin over the damaged endothelium, altered or corkscrew-shaped nuclei in smooth muscle cells, as well as stretching and focal dehiscence in the IEL. The changes in the endothelial cells spontaneously reversed completely by day 7 post-angioplasty, whereas the media and IEL still showed mild residual chronic changes at that time. This study suggested that angioplasty caused reversible damage, and that smooth muscle alteration may be partially responsible for the mechanism of action of TBA in cerebral vasospasm. The structural alterations appeared similar but less severe than those observed in coronary arteries following angioplasty.

In an abstract in 1991, Fujii et al. (408) reported on experimental work performed using the canine double hemorrhage model of vasospasm. Angiographically, angioplasty was most effective at the peak of vasospasm and the dilatation effect was maintained several weeks after the procedure. Pathologically, denudation of endothelial cells and stretching of the internal elastic lamina without disruption of the muscle layer were observed immediately after the angioplasty. These influences cleared within 4 weeks without any sclerotic changes such as intimal hyperplasia. *In vitro* pharmacological study of angioplasty on vasospastic segments showed significant reduction of vascular tension induced by vasoconstrictors. The authors concluded that balloon angioplasty for vasospasm would be most effective at its peak and that the mechanism of continuous dilatation may be slight functional vascular damage affecting muscle tension.

In a comparative study performed in 1991, Yamamoto et al. (363) examined the effects of *in vivo* angioplasty in normal cat femoral arteries, and the effects of *in vitro* angioplasty in cadaveric human middle cerebral arteries. Polyethylene balloons (2.5 x 10 mm when inflated) were used to dilate vessels at 1.5 atmospheric pressure for 30 seconds in one group, and at 3 atmospheric pressure for the same duration in another group. Formic acid digestion of all the cellular components exposed the underlying connective tissue matrix, which was examined with scanning electron microscopy. The authors found that the extracellular collagen fibers and the IEL were slightly stretched

and torn at 3 atmospheric pressure. They concluded that the longlasting effects of balloon dilatation may be caused by the disruption of the connective tissues that proliferate in the vessel wall after a subarachnoid hemorrhage. In 1992, Konishi et al. (403) looked at cadaveric human vasospastic arteries with light and scanning electron microscopy. They found similar findings to those described above.

In 1993, Kobayashi et al. (402) reported on angioplasty of cerebral arteries in a primate model. These experiments examined morphological changes in cerebral vessels after TBA using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Seven days following experimental SAH, vasospastic arteries dilated with a silicone balloon showed minimal endothelial injury, straightening of corrugations in the IEL and endothelium, and intact smooth muscle cells.

In a report in 1993, Benashvili et al. (181) described the architecture of intravascular collagen using SEM in patients with vasospasm before and after angioplasty. The arterial wall in spasm after SAH showed proliferation of connective tissue in the tunica media and tunica intima. After angioplasty there was thinning of the arterial wall by compression and stretching, without disruption of cellular and connective tissue elements and without damage to endothelium. It was concluded that the efficacy of angioplasty was due to compression and stretching of post-SAH proliferated connective tissue.

In 1993, Kobayashi et al. (406) reported further on morphological changes using a monkey model of vasospasm. In this study, SEM of vasospastic arteries demonstrated endothelial convolutions covered with abnormal endothelium along the longitudinal axis. Areas with detached endothelium were observed in the vessels. SEM of the arteries with balloon dilatation showed the convolutions flatter than those of vasospastic arteries. The degree of endothelial cell damage resulting from the angioplasty was slight without additional endothelial injury. TEM of vasospastic arteries demonstrated marked corrugations of the internal elastic lamina, and endothelial cells compressed between tight folds of the elastic lamina, and a small number of smooth muscle cells with changes. TEM of the arteries with balloon dilatation showed that the corrugated internal elastic lamina was extended. The degree of the endothelial cell damage resulting from the

angioplasty was not so severe as that due to vasospasm itself. Most smooth muscle cells appeared intact following angioplasty.

In 1995, Honma et al. (409) used light and electron microscopy to study the morphological changes in cerebral arteries after percutaneous transluminal angioplasty (PTA) for vasospasm in two patients with aneurysmal subarachnoid hemorrhage. In the first patient, postmortem examination of the inflated middle cerebral arteries revealed a heterogeneously extended wall and a dilated lumen. Throughout the vessel wall the extracellular matrix, which was composed of nonmuscle components such as collagen, was stretched in conjunction with the medial muscle component. Also, torn and thinned areas of the wall and intramural hemorrhages were caused by overinflation. In the second patient, prominent stretching of the walls at the atheromatous plaque margin in the dilated vessel was found in addition to the morphological changes observed in the first patient. The authors concluded that characteristic pathological alterations might be present in the vessel wall at the site of angioplasty. The major mechanism of the long-lasting effects of PTA were concluded to be the stretching and disruption of both the degenerative muscle and the proliferative nonmuscle components, mainly in the media of the vasospastic vessels.

In 1995, Macdonald et al. (231) used a rabbit cervical carotid artery model of vasospasm to study the pathological effects of angioplasty. Specimens of nonangioplastied vasospastic and angioplastied vasospastic vessels were compared 1 day, 7 days, and 3 to 4 weeks post angioplasty. Blinded, semiquantitative histopathological study of the arteries showed that 3 to 4 weeks after angioplasty, there was significant endothelial proliferation and a trend for thinning of the tunica media. Angioplasty was not associated with significant arterial fibrosis as measured by hydroxyproline content. The authors concluded that the increase in endothelial proliferation and decrease in the thickness of the tunica media suggested angioplasty damaged endothelial and smooth muscle cells and that this may be the basis for the observation that vasospastic arteries do not re-constrict after angioplasty.

In another study reported in 1995, Macdonald et al. (246) used the same rabbit model of vasospasm to study the pharmacological effects of angioplasty (which was

performed two days after placement of blood clot around the vessel). Vessels were studied 1 day, 5 days, and 28 days after angioplasty. On day 1 and day 5, arteries had significantly reduced contractions to serotonin, KCl, and caffeine when compared to controls. On day 28, contractions were reduced in vasospastic arteries when compared with controls; there were no differences between angioplastied arteries and controls. On all study days, vasospastic arteries had significantly decreased acetylcholine-induced relaxations (after contraction with serotonin) when compared with normal arteries. Arterial wall compliance was significantly decreased in the vasospasm and control groups at all times after angioplasty, although there were no significant differences between arteries with and without angioplasty. The authors concluded (1) that the arteries do not re-constrict after angioplasty because angioplasty decreases smooth muscle contractility and (2) that there is no evidence that angioplasty disrupts the arterial wall matrix, as judged by the lack of increase in arterial wall compliance after angioplasty.

Also in 1995, Chan et al. (357) reported on the pharmacological and morphological effects of immediate *in vitro* transluminal balloon angioplasty on normal and vasospastic canine basilar arteries. It was found that immediately after TBA of both normal and vasospastic arteries, there was a significant reduction of responses to the vasoconstrictors (KCl, noradrenaline, serotonin, and prostaglandin $F_{2\alpha}$) and vasorelaxants (calcium ionophore A_{23187} and bradykinin) used. As revealed by scanning EM and transmission EM, both normal and vasospastic vessels dilated with TBA showed flattening and patchy denudation of the endothelium, and straightening and occasional rupture of the internal elastic lamina. In addition, vasospastic vessels dilated with TBA showed decreased surface rippling and mild stretching and straightening of smooth-muscle cells, and mild thinning of the tunica media. There was no gross vascular disruption or obvious change in the extracellular matrix of the vessel walls of either normal or vasospastic arteries after TBA. The authors concluded that these results were suggestive that a functional impairment of vasoreactivity in the vessel wall, as a result of mechanical stretching of the smooth-muscle layer, played a more important role than structural alteration in the immediate dilation produced in vasospastic arteries by TBA.

Also in 1995, Fujii et al. (407) studied the *in vivo* effects of transluminal balloon angioplasty on the canine basilar artery. TBA was performed using catheters specially designed to be navigated into the small canine basilar artery (401,410-412). TBA was performed 1, 4, or 7 days after SAH induction; it was also performed on normal vessel segments. Angiography and light microscopy of vessel segments were performed on days 7, 8, 14, and 50, while pharmacological responses to vasoconstrictors (KCl and thromboxane analogue A_2 [U46619]) were determined on day 7 after angioplasty. For dogs undergoing TBA on day 1 or day 4, angiography on day 7 showed that there was no significant difference between the diameters of angioplastied arteries and vasospastic arteries and the authors concluded that in this model of vasospasm TBA did not have a "preventive" effect. When arteries were dilated on day 7 there was no angiographic recurrence of vasospasm either immediately after the procedure or on days 8, 14, or 50. Histologic examination of arteries with light microscopy revealed characteristic changes in vasospastic arteries, including partial denudation of endothelial cells, hypertrophy of the tunica media, and folding of the internal elastic lamina. Immediately after TBA there was denudation of the endothelial cells, thinning of the intima, extension of the internal elastic lamina and some disruption of the muscle layer. However, desquamation and splitting of the tunica media or internal elastic lamina were not observed. The findings on day 8 were similar to those on day 7; by day 50 the endothelium, internal elastic lamina, and tunica media all showed nearly normal structures with no residual deleterious effects of TBA. Normal vessel segments and vessel segments undergoing TBA in the absence of SAH did not show any difference in their responses to vasoconstrictors on day 7. On the other hand, vessel segments undergoing TBA after SAH showed significantly diminished responses compared to normal vessels when exposed to the vasoconstrictors on day 7. The authors concluded that TBA had a more pronounced effect on vasospastic vessels than on normal vessels, that the mechanism of TBA vasodilatation in vasospasm after SAH may result from mild functional changes in the vascular wall when TBA was performed on day 7 after SAH, and that the functional changes could not be adequately induced to prevent recurrence of vasoconstriction when TBA was applied soon after SAH.

The studies described above suggest that morphological changes found after TBA in normal and vasospastic cerebral arteries can vary from mild to severe. In extreme cases, all three layers of the vessel wall may be damaged. However, most studies showed only mild changes consisting of flattening of endothelium without any tearing, straightening of the IEL with occasional focal dehiscence from the underlying tunica media, and only minimal changes in the tunica media itself. Variability between studies is probably related to the extent, duration and pressure of dilatation, the type of balloon catheter used, as well as interspecies and anatomical differences. Polyethylene and latex balloons are designed for coronary or peripheral vessel angioplasty in which a great deal of tension or pressure needs to be generated by the balloon to disrupt an atherosclerotic plaque and dilate an artery narrowed by such a plaque (373). There is a high incidence of complications, such as vessel wall rupture, arterial thrombosis and occlusion associated with the use of such balloons in coronary angioplasty (373). Acute occlusion or re-stenosis after coronary angioplasty occurs in 2-4% of cases (373). This is mainly due to either acute vasospasm, thrombosis or plaque dehiscence and dislodgement, causing occlusion (369-371,376-377,380). A damaged luminal surface predisposes to platelet adhesion and thrombi formation with release of vasoactive agents causing vasospasm (373). Re-stenosis after successful coronary angioplasty occurs in up to 30% of cases, usually in the first 6 months after angioplasty (373). This is most commonly seen in cases in which the initial stenosis is severe, or in which there is still a high grade of stenosis after the initial angioplasty. Silicone elastomer balloons, on the other hand, are much softer and flexible and elongate and conform to the luminal contour of the vessel. At present, human cerebral angioplasty is routinely performed using silicone balloons. The pressure generated (usually 50.7-152 kPa or 0.5-1.5 atmospheres) by this type of balloon is usually much less than that achieved with polyethylene or latex balloons (363).

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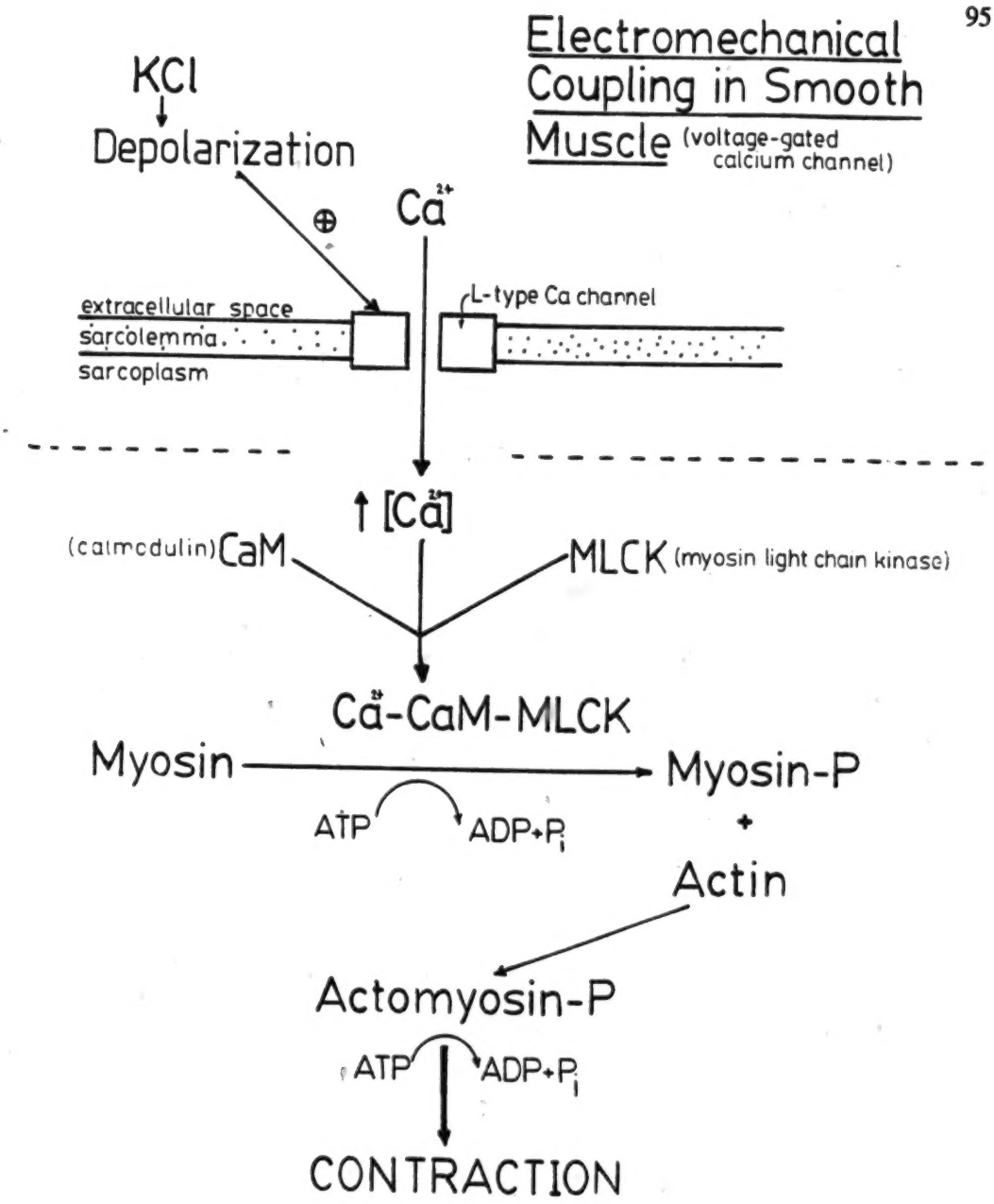

Figure I-1
Electromechanical coupling in smooth muscle (voltage-gated calcium channel) and intracellular mechanism of smooth muscle contraction. For detailed description see pages 7 to 9 in the text.

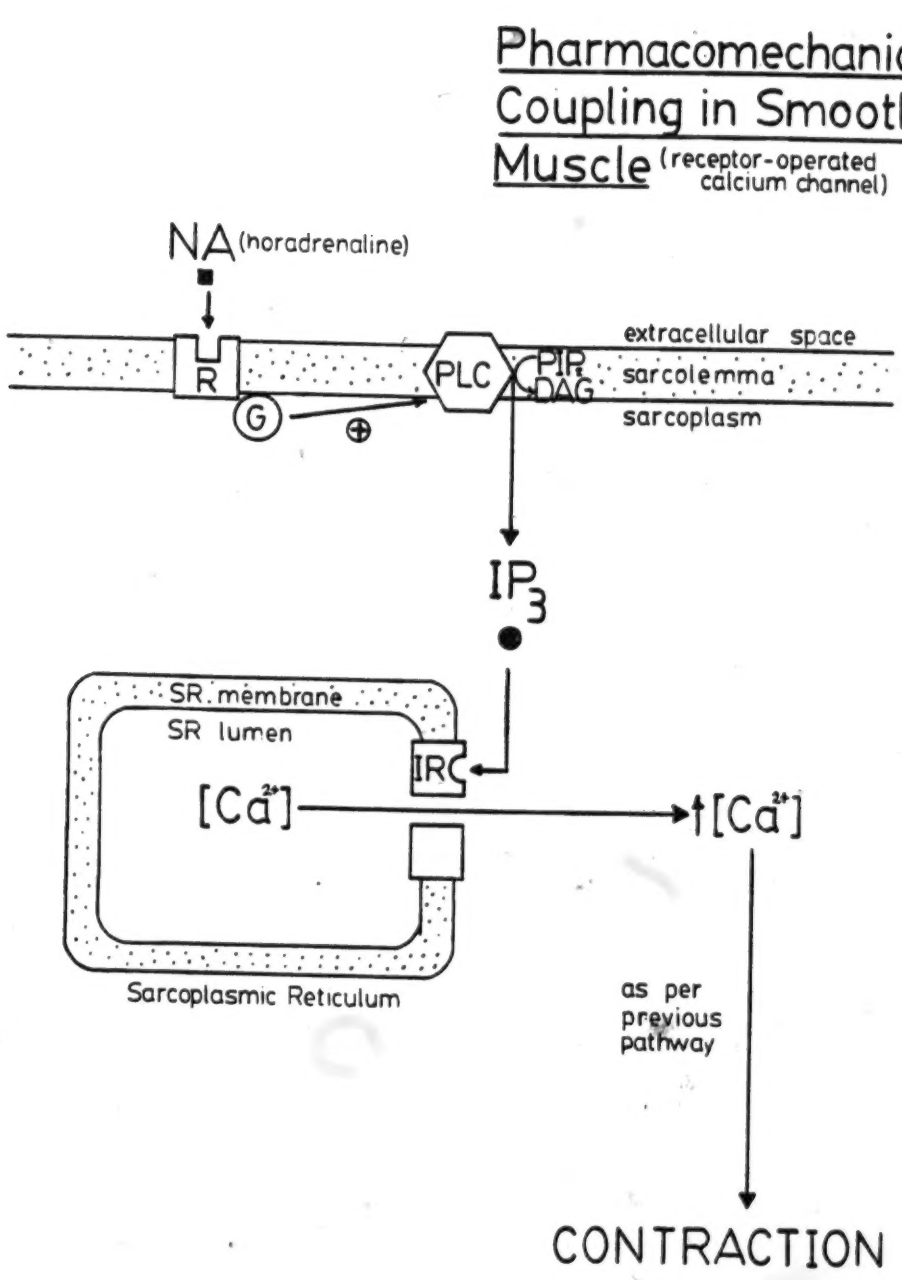

Figure I-2
Pharmacomechanical coupling in smooth muscle (receptor-operated calcium channel) and mechanism of release of calcium from the sarcoplasmic reticulum. Previous pathway refers to Figure I-1. For detailed description see pages 7 to 9 in the text.

RELAXATION
(Endothelium-dependent)

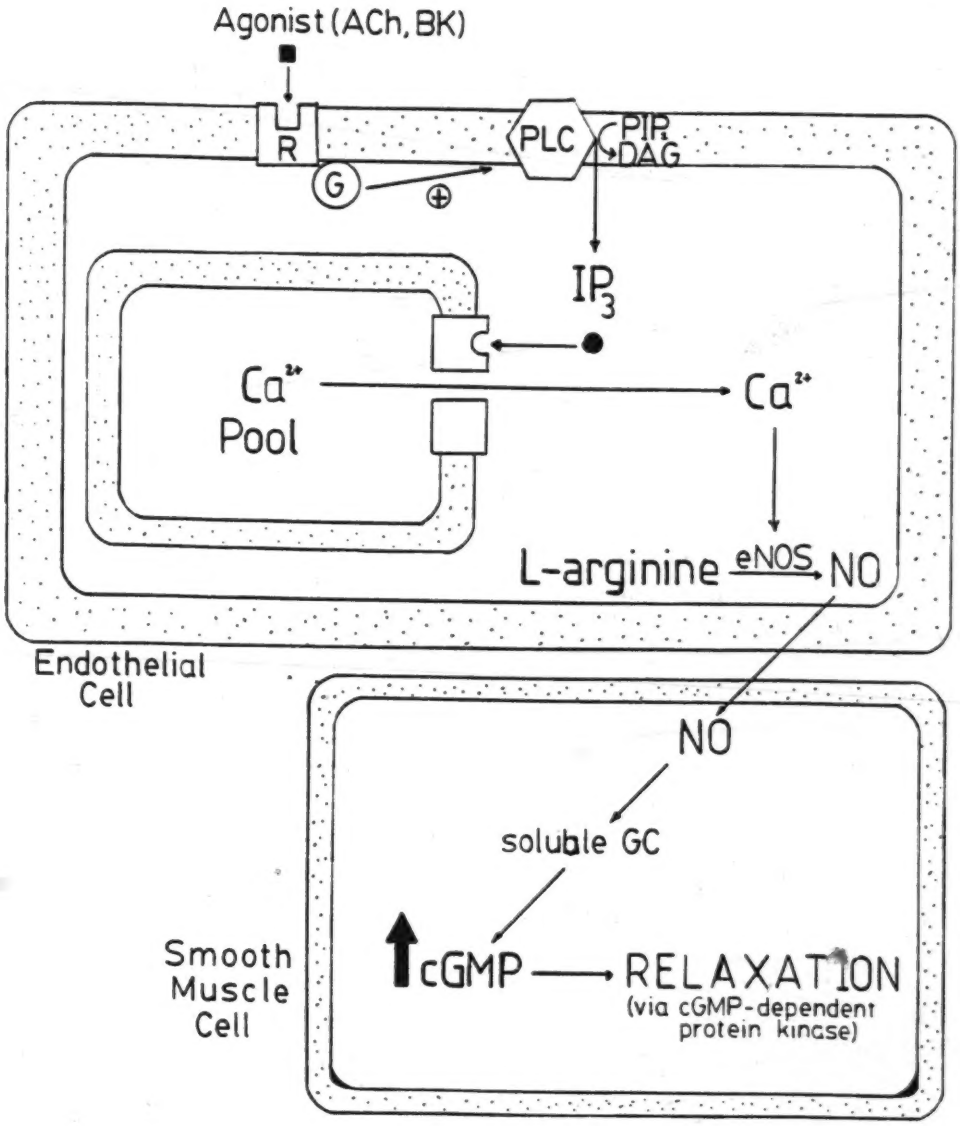

Figure I-3
Intracellular mechanisms involved in the endothelium-dependent relaxation of vascular smooth muscle cells. For detailed description see pages 11 to 14 in the text.

CONTRACTION
(via Endothelin)

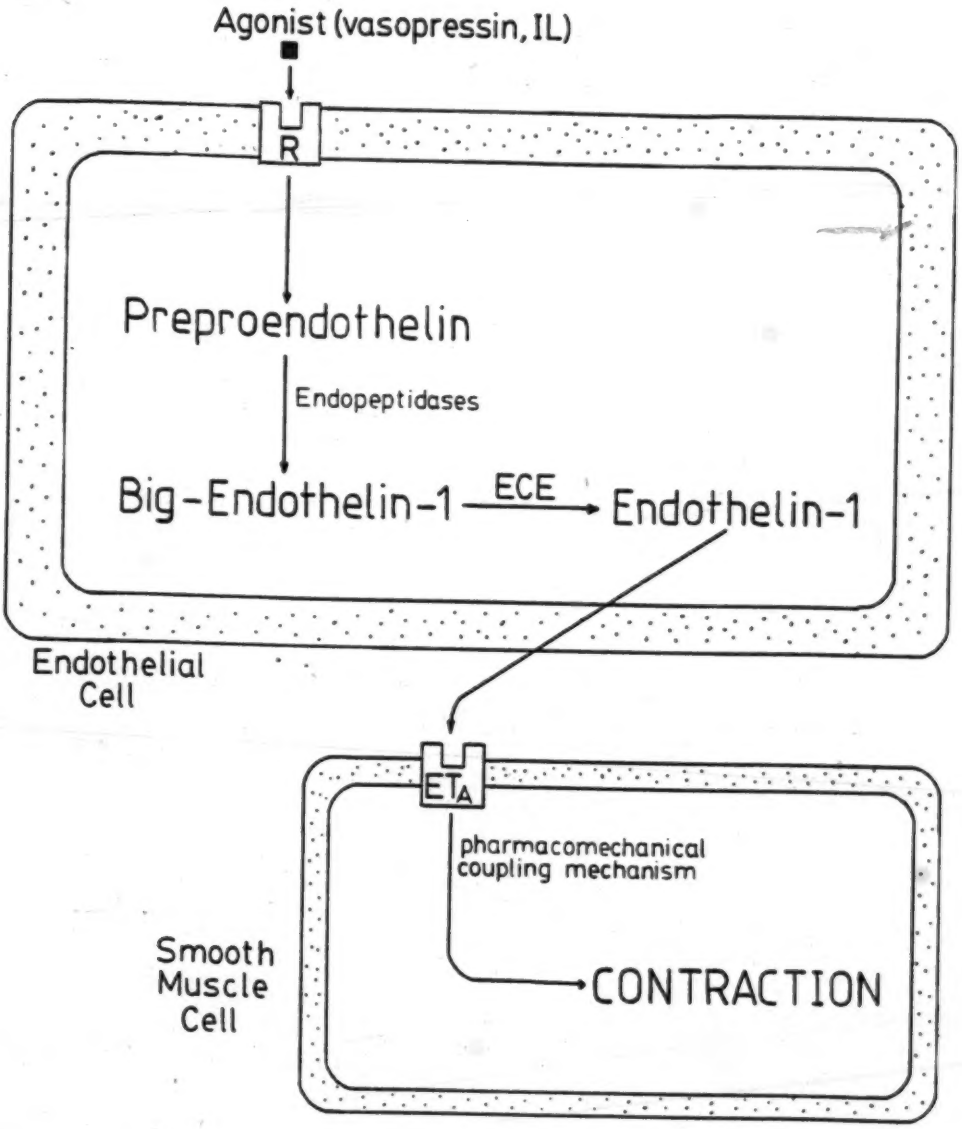

Figure I-4
Intracellular mechanisms involved in the production of endothelin-1 in endothelial cells and its action on smooth muscle. For detailed description see pages 15 to 18 in the text.

VASOSPASM PATHWAYS
(Smooth Muscle Cells)

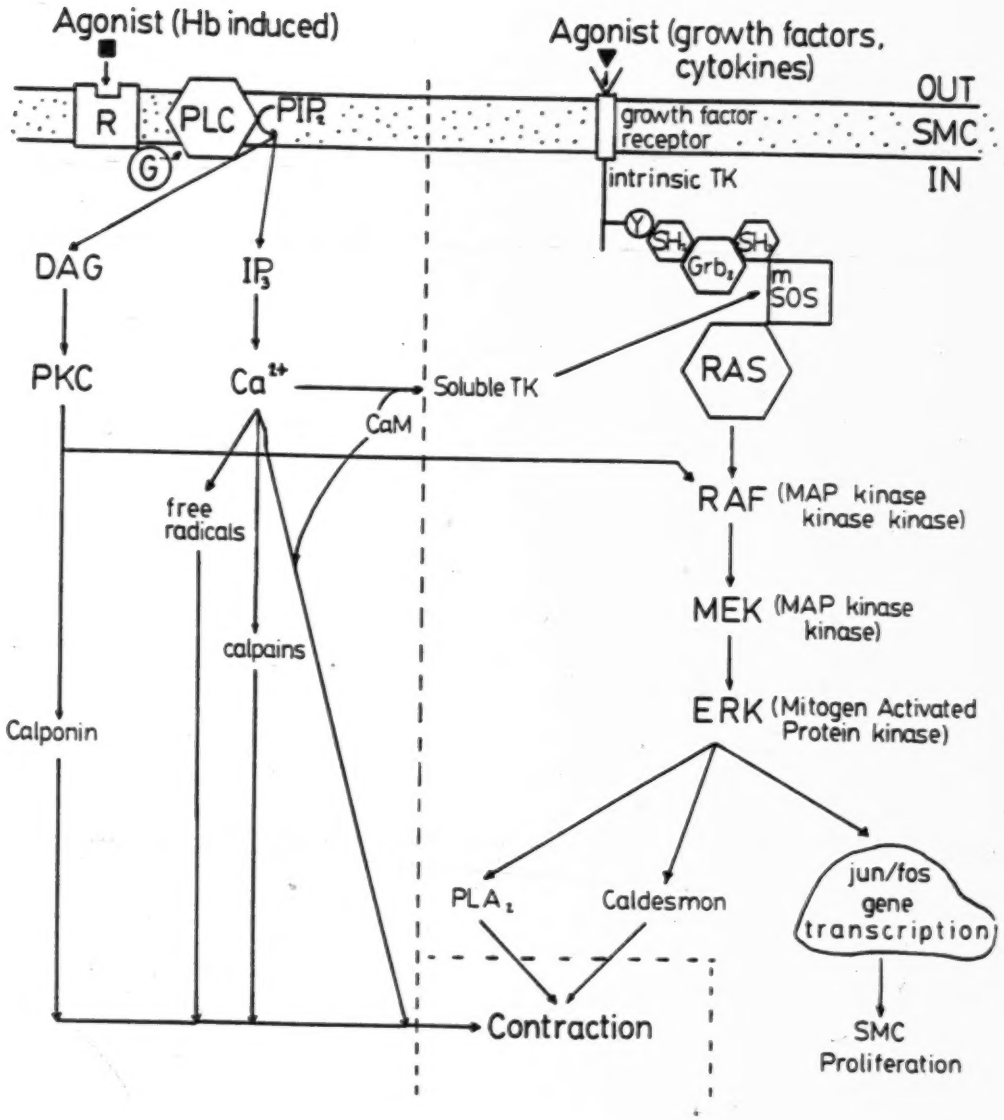

Figure I-5
Possible intracellular pathways in smooth muscle cells that may lead to vasospasm in cerebral arteries. For a detailed description see text under "Smooth muscle contraction" and "Pathogenesis of cerebral vasospasm", with special attention to pages 24 to 25.

CHAPTER TWO

IN VIVO ANIMAL MODELS OF SUBARACHNOID HEMORRHAGE AND VASOSPASM: DEVELOPMENT OF A NEW CANINE CAROTID ARTERY MODEL OF VASOSPASM AND REVIEW OF THE LITERATURE

INTRODUCTION

Ideally, studies on the pathogenesis and treatment of cerebral vasospasm in humans should be conducted using human cerebral arteries. *In vivo* experiments on humans are of course ethically unacceptable and post mortem pathological examination of human arteries in vasospasm can only provide a finite amount of information. This has led to the development of animal models of vasospasm.

Since it is believed that the occurrence and rupture of cerebral aneurysms in animals is very rare, there are no naturally occurring animal models of subarachnoid hemorrhage (SAH) and vasospasm. Accordingly, a number of animal models have been developed which endeavor to simulate the conditions necessary for vasospasm to develop. In general these models use one of two techniques: (1) an artery is punctured allowing blood to escape and collect around the artery and its neighbors or (2) an artery is surgically exposed and autologous blood obtained from another site is placed around the artery. Each of these techniques has its advantages and disadvantages.

The majority of animal models of SAH and vasospasm use intracranial arteries because it is believed that these vessels most closely resemble the intracranial vessels of humans. Recently, however, extracranial arteries have been successfully used in vasospasm experiments. This report describes a new model of vasospasm that uses the high cervical internal carotid artery (ICA) of dogs and discusses it in the context of other animal models of SAH and vasospasm.

MATERIALS AND METHODS

Eight mongrel dogs of either sex weighing between 15 and 26 kg were use for this study. The protocol was evaluated and approved by the University of Alberta

Animal and Ethics Review Committee and experiments were conducted with strict adherence to the standards of the Canadian Council on Animal Care. The protocol is summarized in Figure II-1.

Surgical Procedure

On Day 0 animals were premedicated with buprenorphine 0.01 mg/kg, ketamine 6 mg/kg, and atropine sulfate 0.02 mg/kg intramuscularly. An intravenous line was inserted in either foreleg with an 18-gauge angiocatheter and Ringer's lactated solution was used for hydration. Animals were anesthetized with sodium pentobarbital 0.5 mg/kg intravenously and intubated. Intravenous boluses of pentobarbital 0.05 mg/kg were given as necessary to maintain adequate anesthesia. Animals were allowed to breath room air spontaneously. In preliminary studies involving several animals arterial blood gas analyses showed that normocarbia was maintained using this technique.

Animals were positioned supine on the operating table and the anterior aspect of the neck was shaved, cleansed with a proviodine solution and draped using aseptic technique (Figure II-2). A midline longitudinal incision was made through the skin over the anterior aspect of the neck using a number 10 blade. Anterior muscles were divided and dissection was carried down so that the trachea was exposed in the midline (Figure II-3). Using blunt dissection the soft tissues on either side of the trachea were divided and the high cervical ICAs exposed bilaterally. Each ICA was catheterized at the proximal aspect of the incision with a 24-gauge angiocatheter and angiography was performed with 5 mL of iothalmate meglumine injected at a rate of 0.75 mL/sec. With the operating microscope used for magnification, 60 mm sections of both ICAs were dissected free of adjacent tissues and any loose adventitia removed (Figure II-4).

One of ICAs (chosen by block randomization) was placed into an empty silicone elastomer cuff which had a 10 mm internal diameter and was 50 to 60 mm in length (Figure II-5). The other ICA was also placed into a silastic elastomer cuff which was then filled with 10 mL of autologous clotted arterial blood that had been obtained via a femoral artery puncture (Figure II-6). Small cotton pledgets were positioned at either end of the tube to contain the clot. The silicone elastomer cuff on each side was secured

with three silk ties along its length (Figure II-7). The soft tissues were then allowed to fall back over the exposed ICAs and the muscles were repositioned over the trachea. Subcutaneous tissues were closed using a running 3-0 Vicryl suture and the skin was closed using a running 2-0 Prolene suture. Animals were monitored carefully in a close observation facility for 24 hours and buprenorphine 0.01 mg/kg was administered as needed for analgesia. After ensuring the recovery of an adequate level of consciousness and the return of the gag reflex the animal was extubated.

The animals were cared for in the usual fashion with free access to food and water and with daily neurological monitoring. On Day 7 the dogs were again anesthetized and intubated as described above. The midline longitudinal incision was reopened, both ICAs were re-exposed and angiography repeated. The animals were then sacrificed using sodium pentobarbital (30 mg/kg). Both ICAs were removed en bloc (Figure II-8).

Both ICAs were placed in Krebs' solution of the following composition: 120 mmol/L NaCl, 5 mmol/L KCl, 1.5 mmol/L $CaCl_2$, 1 mmol/L KH_2PO_4, 1 mmol/L $MgSO_4$, 25 mmol/L $NaHCO_3$, 55 mmol/L dextrose, aerated with 95% O_2 / 5% CO_2 and maintained at 37°C. These arterial segments were used for pharmacological studies and morphological studies (electron microscopy). In addition, a segment of ICA proximal to the original surgical field and the previously dissected ICA segment was removed on one side from each animal to provide a control preparation for both the pharmacological and morphological studies. Two rings were cut from each isolated ICA, as well as the control segment, enabling the pharmacological study of cuffed blood coated ICAs, cuffed non-blood coated ICAs and normal control ICAs. The inner diameters of the segments were measured with a micrometer under magnification before pharmacological analysis. The remainder of the arterial segments were prepared for morphological analysis

Angiographic measurement of vasospasm and the effects of TBA

For all angiograms the diameter of the ICA in millimeters was determined by direct measurement of the angiogram, at the point corresponding to the midpoint of the blood clot-filled or empty silicone elastomer cuff. For all groups, the degree of

angiographic vasospasm (if any) was determined by comparing vessel diameters on Day 0 with vessel diameters on Day 7 and calculating a percentage change in vessel diameter.

Pharmacological Studies

Responses of arterial rings were recorded isometrically using force-displacement transducers connected to a polygraph (Figure II-9). Rings of cerebral arteries were suspended between two stainless-steel hooks, under a resting tension of 1 gram in organ baths of 10 mL working volume containing Krebs bicarbonate solution maintained at 37°C and bubbled with 95% O_2 / 5% CO_2. After an equilibration period of 1 hour, during which the Krebs solution was changed every 15 minutes, the response to potassium chloride (KCl, 60 mmol/L) was recorded and preparations were washed until resting tension was again obtained. Cumulative dose-response curves for noradrenaline (10^{-9} to 10^{-5} mol/L) and serotonin (5-HT, 10^{-9} to 10^{-5} mol/L) were then recorded for each arterial ring. Tonic contraction of preparations was established using noradrenaline (10^{-5} mol/L) prior to vasorelaxation studies in which cumulative dose-response curves to the calcium ionophore A_{23187} (10^{-8} to 10^{-5} mol/L) were obtained. The response to papaverine (5×10^{-4} mol/L) was also recorded after tonic contraction using noradrenaline (10^{-5} mol/L) had been established. Preparations were washed until the resting tension had been restored, before another agent was tested. The ring preparations were studied in the organ baths for approximately 8-10 hours. At the end of each experiment the ring preparations were tested with KCl (60 mmol/L) to confirm that the responses were not different from the initial responses obtained to KCl at the beginning of the experiment.

Electron Microscopy

Segments of vessels were examined with transmission electron microscopy and scanning electron microscopy. Cross-sections of the vessel wall were examined with transmission electron microscopy. The intact vessel wall, luminal and cross-sectional aspects, were examined with scanning electron microscopy.

All specimens were prefixed in 2.5% glutaraldehyde in 0.12 mol/L Millonig's buffer solution (pH 7.2) overnight at room temperature. After samples had been washed

three times for 15 minutes each in Millonig's buffer, they were postfixed with 1% osmium tetroxide (OsO_4) in the same buffer for 2 hours. These samples were briefly washed in distilled water, and dehydrated in a graded series of ethanol solutions (50%, 60%, 70%, 80%, and 90%: 10-15 minutes each grade) before the final two 10-minute rinses with absolute ethanol. From this point onward, preparation for scanning electron microscopy and transmission electron microscopy samples differed.

For the scanning electron microscopy study, samples in absolute ethanol were dried in a CO_2 critical-point drier at 31°C for 5-10 minutes, and then mounted on aluminum stubs. All samples were sputter-coated with gold prior to examination under the scanning electron microscope.

For the transmission electron microscopy study, absolute ethanol bathing the samples was replaced with propylene oxide, which was changed three times at 10-minute intervals. Samples were then embedded in Araldite CY212 mixture/propylene oxide for 1 hour, and subsequently in complete Araldite CY212 mixture overnight in a vacuum desiccator. The mixtures embedding the samples were allowed to polymerize at 60°C for 2 days prior to sectioning. Sections were stained with 4% uranyl acetate for 30 minutes and lead citrate for 5 minutes. Photomicrographs of samples were taken at 75 kV using a transmission electron microscope.

Morphological appearance of cuffed blood coated, cuffed non-blood coated, and normal control arteries were assessed separately; that is, cuffed blood coated arteries and cuffed non-blood coated arteries were compared to normal arteries, respectively. Based on electron micrographs, a pair-wise semi-quantitative comparison of morphological differences between cuffed blood coated and normal arteries and cuffed non-blood coated and normal arteries for each dog was performed. Specific features in scanning electron micrographs of the intact vessel wall were identified as follows: degree of luminal narrowing, wall thickness, and corrugation of the tunica intima and internal elastic lamina (IEL). For transmission electron microscopy of vessel cross-sections, features looked for (in addition to those above) included alterations of endothelial cells in the tunica intima (rounding and folding) and alterations of smooth muscle cells in the tunica media (rounding and surface rippling).

Statistical Analysis

For the pharmacological study, comparisons between groups at each concentration for each vasoconstrictor or vasodilator was assessed using one way analysis of variance (ANOVA), followed by a Scheffe's test of multiple comparisons if a significant probability was reached. The same method was used to compare group data of vessel lumen diameters measured with a micrometer. For the angiographic study, comparisons within a group over time were made using a paired t-test. Data were expressed as mean ± standard error (SE) of the mean. A probability level of less than 0.05 was considered significant.

Equipment and Supplies

Force-displacement transducers (model FT.03) and a polygraph (model 7D) were obtained from Grass Instrument Co., Quincy, Massachusetts. The critical-point dryer was manufactured by Seevac, Inc., Hialeah, Florida. The sputter coater, model S150B, was manufactured by Edwards Vacuum, West Sussex, England. The scanning electron microscope (model S-2500) and the transmission electron microscope (H-7000) were obtained from Hitachi Ltd., Tokyo, Japan.

RESULTS

Angiographic Measurement of Vessel Diameter

The placement of a clot of autologous blood contained within a silicone elastomer cuff around the ICA resulted in a highly significant constriction of the artery. On Day 7 after clot placement the angiographic vessel diameters were 33% to 85% of their original size, with a mean reduction to 55±6% ($P<0.05$) (Figure II-10B) . The placement of an empty silicone elastomer cuff around the ICA did not result in a significant constriction or dilatation of the artery. On Day 7 after cuff placement the angiographic vessel diameters were 91% to 129% of their original size, with a mean of 102±4% ($P>0.05$) (Figure II-11B). Similar results were obtained when the actual vessels were measured with a micrometer. On Day 7 after placement of a blood clot in a silicone elastomer cuff, the measured vessel diameters were 66% to 77% the size of

normal vessels, with a mean of 72±2% ($P<0.05$). On Day 7 after placement of an empty silicone elastomer cuff, the measured vessel diameters were 88% to 108% the size of normal vessels, with a mean of 100±2% ($P>0.05$).

Pharmacological Effects of Vasoconstrictor Agents

Responses to a single dose of KCl (60 mmol/L) and cumulative dose-response curves for noradrenaline and 5-HT were recorded for the three groups of vessels: normal, cuffed blood coated, and cuffed non-blood coated.

KCl

Vessels surrounded by an empty cuff did not show a significantly different response in comparison to normal vessels when exposed to KCl at 60 mmol/L. The response of cuffed blood coated vessels was diminished when compared to cuffed non-blood coated vessels and normal vessels, but not at a significant level. These results are shown in Figure II-12A.

Noradrenaline

Vessels surrounded by an empty cuff did not show a significantly different response in comparison to normal vessels when exposed to noradrenaline at any concentration. The response of cuffed blood coated vessels was diminished when compared to cuffed non-blood coated and normal vessels at all concentrations, but not at a significant level. These results are shown in Figure II-12B.

5-HT

Vessels surrounded by an empty cuff did not show a significantly different response in comparison to normal vessels when exposed to 5-HT at any concentration. The response of cuffed blood coated vessels was diminished when compared to cuffed non-blood coated and normal vessels at all concentrations, but not at a significant level. These results are shown in Figure II-12C.

Summary

Vessels surrounded by an empty cuff exhibited the same responses as normal vessels when tested with each vasoconstrictor at each concentration. Cuffed blood coated vessels (vasospastic) exhibited diminished responses as compared to normal vessels and cuffed non-blood coated vessels when tested with each vasoconstrictor at each concentration.

Pharmacological Effects of Vasodilators

After tonic contraction with noradrenaline (10^{-5} mol/L) had been established, cumulative dose-response curves for the calcium ionophore A_{23187} and the response to a single dose of papaverine (5×10^{-4} mol/L) were recorded for the three groups of vessels.

Calcium Ionophore A_{23187}

Vessels surrounded by an empty cuff did not show a significantly different response to normal vessels when exposed to the calcium ionophore A_{23187} at any concentration. The response of cuffed blood coated vessels was diminished when compared to cuffed non-blood coated and normal vessels at all concentrations but not at a significant level. These results are shown in Figure II-13.

Papaverine

All vessel groups showed 100% relaxation after exposure to papaverine.

Summary

Vessels surrounded by an empty cuff exhibited the same responses as normal vessels when tested with the calcium ionophore A_{23187}, an endothelium-dependent vasorelaxant, at each concentration. Cuffed blood coated vessels (vasospastic) exhibited diminished responses as compared to normal vessels and cuffed non-blood coated vessels when tested with the calcium ionophore A_{23187} at each concentration. Not unexpectedly vessels in all groups exhibited maximal vasorelaxation when exposed to papaverine.

Changes Observed With Scanning Electron Microscopy

Scanning electron microscopy of normal and cuffed non-blood coated vessels did not reveal any differences. Photomicrographs of normal, cuffed non-blood coated, and cuffed blood coated (vasospastic) vessels are shown in Figure II-14. Close up photomicrographs of normal and vasospastic vessels are shown in Figure II-15. Scanning electron microscopy of vasospastic vessels showed moderate diminution of the vessel lumen, decreased luminal diameter/wall thickness ratio (Figure II-14C and Figure II-15B, bottom), corrugation of the internal elastic lamina, and folding of the endothelial surface (Figure II-15B, top).

Changes Observed With Transmission Electron Microscopy

Transmission electron microscopy of normal and cuffed non-blood coated vessels did not reveal any differences. Photomicrographs of normal and cuffed blood coated (vasospastic) vessels are shown in Figure II-16. Results were consistent with those obtained with scanning electron microscopy; vasospastic vessels showed corrugation of the internal elastic lamina, folding of the endothelial surface, and thickening of the vessel wall, especially the tunica media (Figure II-16B). There was some swelling and vacuolation of endothelial cells, with cellular rounding and some cell separation. Rounding of smooth muscle cells with surface rippling and occasional breaks in the internal elastic lamina were also noted.

DISCUSSION

A certain amount of information may be obtained by the post-mortem evaluation of arteries taken from humans who have died while suffering cerebral vasospasm (1-5). However, since the experimental study of living human cerebral vessels is impossible, animal models have been developed for this purpose. *In vitro* study of SAH and vasospasm in animals involves the removal of the artery to be tested immediately after the animal has been sacrificed (6-10). The artery is then held within a fixation device and subjected to various test substances which may include autologous blood. The artery is observed to determine if contraction or relaxation occurs and then various

physiological, pharmacological and morphological properties can be studied, depending on the focus of the experiments. Using *in vitro* techniques, putative spasmogens have been studied (6,10,11), as well as potential vasospasm therapies (12,13).

Since *in vitro* experiments require that arteries be removed from their natural environment and be subjected to artificial manipulation there are limitations as to the amount of information that may be obtained from such studies. For example, long-term experiments are not possible using *in vitro* systems. Accordingly, *in vivo* models of SAH and vasospasm have been developed and proposals for ideal experimental models put forth (14-16). At minimum an *in vivo* model should have as its fundamental component a blood clot surrounding a vessel in a living animal. This blood clot should result in consistent and reproducible delayed vasoconstriction that lasts for several days. In better models the vasoconstriction should be angiographically detectable, the time course of vasospasm development and resolution should be similar to that observed in humans, and animal survival should be such that efficacy and toxicity studies of therapeutic modalities are possible. In the ideal model the experimental animal should be as similar to humans as possible and the SAH should be created in manner that most closely resembles an aneurysmal rupture. Other desirable features of *in vivo* models are that they be relatively easy to produce and that they be cost effective.

Since there are no naturally occurring animal models of vasospasm a number of techniques have been used to deliberately produce SAH in animals which subsequently leads to the development of delayed-onset, sustained arterial narrowing. These techniques generally fall into one of two categories: (1) an artery is punctured allowing blood to escape and collect around the artery and its neighbors or (2) an artery is surgically exposed and autologous blood obtained from another site is placed around the artery. Each of these techniques has its advantages and disadvantages. One common feature to all animal models of SAH and vasospasm is that the animals very rarely develop a vasospasm-related ischemic neurological deficit (17). This is probably because the brains of animals are provided with an abundance of collateral blood flow. Since most vasospasm experiments are studying vessel constriction and how it can be reversed,

rather than vasospasm-related ischemia, the absence of ischemic neurological deficits in experimental animals is probably of little significance. A historical perspective on *in vivo* animal models of SAH and vasospasm follows. Only animal models of vasospasm in which cerebral arteries are rendered vasospastic *in vivo* and not models where normal vessels are harvested and tested *in vitro* are included in this review. Selected models of SAH and vasospasm are presented in Table II-1. General features of the models are presented in Table II-2.

In Vivo Models of SAH and Vasospasm Using Intracranial Arteries

The advantages of models using intracranial arteries include the presence of a cisternal space into which autologous blood can be introduced and retained, and comparisons with human SAH and vasospasm are probably more valid. *In vivo* models using intracranial arteries have been developed in rats, rabbits, cats, pigs, dogs and primates.

Rat

In 1979 Barry et al. reported on the first use of the rat in an experimental model of vasospasm (18). Anesthetized animals were intubated endotracheally and then under the microscope a tungsten microelectrode was stereotactically positioned above the basilar artery via an approach through the clivus. The artery was punctured and the ensuing SAH visualized. The degree of vasospasm was subsequently determined by direct observation. Vasospasm was significant on the second postoperative day but had resolved by the third, by which time the blood in the subarachnoid space had also cleared.

In 1983 Lacy and Earle described how they drilled a small hole through the frontal bone of the rat cranium through which a cannula could be passed to allow the introduction of blood into the area around the circle of Willis (19). However, since these authors were studying electrocardiographic abnormalities after SAH, they did not measure vessel diameter so it is not possible to determine the degree of vasospasm

induced by this method.

In 1985 Solomon et al. described how to use the operating microscope to make a small hole through the occipital bone of the rat, exposing the atlanto-occipital membrane, and how to stereotactically position a cannula into the cisterna magna through which autologous blood could be injected (20). Actual vessel diameter was not measured but rats subjected to a SAH showed a 40% decrease in hemispheric cerebral blood flow (using the radioactive microsphere technique) which the authors interpreted as being indicative of vasospasm. This model or slight modifications have been used for subsequent studies on vasospasm (21-25).

Also in 1985, Delgado et al. reported on a slightly different technique to place an indwelling catheter into the cisterna magna of rats through which homologous blood could be injected (26). Using angiography they observed a biphasic pattern of vasospasm with maximal acute spasm at ten minutes and maximal late spasm at two days after cisternal injection. This model has been used in subsequent studies of vasospasm (27-31), as has a slightly modified form in which a small gauge needle is used to inject blood into the cisterna magna (32-36).

In 1986 Doczi et al. described how they injected autologous blood via burr holes into various locations around the brain: the right cerebral convexity, the right lateral ventricle, the basal cisterns (by piercing the soft palate and skull base) and the cisterna magna (37). However these authors were studying blood-brain barrier changes during SAH and did not measure vessel diameter in order to determine the degree of vasospasm present.

In 1990 Kader et al. reported a modification of the technique of Barry et al. (18) and showed that 3, 7, and 14 days after SAH there was a significant reduction in telencephalic cerebral blood flow (as measured by the [^{14}C]butanol indicator fractionation technique) which the authors interpreted as being indicative of vasospasm (38). Also in 1990 Barth et al. described how they had subjected rats to middle cerebral artery occlusion via a craniectomy 3 days after transclival basilar artery exposure (39). This was also a modification of the model of Barry et al. (18) and resulted in rats with a symptomatic delayed neurological deficit, which the authors attributed at least partly to

vasospasm.

In 1995 Bederson et al. reported on a model in which they advanced a 3-0 monofilament suture centripetally via the external carotid artery into the intracranial internal carotid artery until resistance was felt (40). The suture, which had a sharpened end, was then pushed 3 mm further penetrating the internal carotid artery near its intracranial bifurcation. The suture was withdrawn and blood allowed to flow into the subarachnoid space. Cerebral perfusion pressure was observed to be diminished up to 24 hours after SAH induction which was interpreted as being representative of the presence of vasospasm.

In 1995 Veelken et al. described how they produced a SAH by passing a nylon thread up through the right internal carotid artery, piercing a hole in the right anterior cerebral artery (41). Three different surgical techniques to accomplish this were tried. A reduction in cerebral blood flow was observed up to 3 hours after the induction of SAH.

In 1995 Piepgras et al. reported a procedure in which they punctured the prechiasmatic cistern via the orbit and optic foramen and injected autologous blood into the subarachnoid space using a small gauge needle (42). A significant arterial narrowing was observed 2 days after SAH induction.

Rabbit

In 1969 Offerhaus and van Gool were the first to report on the rabbit as an experimental model of subarachnoid hemorrhage (43). A catheter was passed through an occipital burr hole and was positioned near the orbit using fluoroscopy. Blood from the right carotid artery was allowed to pass directly through the catheter to the subarachnoid space. As the purpose of the experiment was to study electrocardiographic changes after 1 hour, the presence of vasospasm was not determined.

In 1982 Edvinsson et al., described how, through a transorbital approach, they injected autologous blood into the chiasmatic cistern of rabbits via a small gauge needle, with the subsequent production of angiographically visible vasoconstriction (44). This model has been used for subsequent studies of vasospasm (45).

In 1983 Logothetis et al. reported a procedure in which a small craniotomy was made on the left side of the cranial vault near the superior sagittal sinus and coronal suture in the rabbit (46). Using a small gauge needle the posterior branch of the middle cerebral artery and the superior sagittal sinus were sequentially punctured. The animals were followed with videomicroscopy, cerebral blood flow measurements, and electrocorticography for up to 72 hours. Acute and chronic vasospasm was observed.

In 1984 Liszczak et al. described how they injected autologous blood into the cisterna magna of rabbits via a small gauge needle using a method similar to one reported previously to study hydrocephalus (47,48). Morphological changes were described but the degree of arterial narrowing was not reported. In 1984 Chan et al. also reported how they injected autologous blood into the cisterna magna of rabbits that were placed in a head-down position on an inclined plane raised 30 degrees to the horizontal (49). Radiographic vertebrobasilar arterial spasm was observed on the 3rd day post-SAH. This model or ones with slight modifications have been subsequently used for studies on vasospasm (12,50-62). In 1987 Baker et al. described a "two-hemorrhage" modification of the cisterna magna injection technique in which blood was introduced on the 1st and 4th day of the experiment (63). Angiographic vasoconstriction was maximal on the 6th day of the experiment and had become less by the 14th day. This model or slight variations of it have also been used for subsequent studies on vasospasm (64-67).

In 1988 Endo et al. reported on a model of SAH which attempted to cause vasospasm that was also symptomatic (68). The common carotid arteries of rabbits were bilaterally ligated 2 weeks before the induction of subarachnoid hemorrhage in an effort to reduce collateral blood flow to the brain. SAH was induced by a single injection of autologous blood into the cisterna magna. Angiographic vasospasm was present up to 6 days post-SAH and the rabbits were judged to be symptomatic. This model has been used for subsequent studies on vasospasm (69). In 1994 Otsuji et al. reported a "two-hemorrhage" modification of this technique with sequential injections of blood into the cisterna magna being performed two days apart (70).

In 1992 Egemen et al. described how they used a transclival approach to visualize the basilar artery, perforating it with a small gauge needle electrode to cause a SAH (71).

Significant angiographic vasospasm was present 5 days post-SAH.

In 1994 Diringer et al. reported on a study part of which compared a single injection of blood into the cisterna magna with transorbital injection of blood into the chiasmatic cistern (72). No difference in the degree of angiographic vasospasm or cerebral blood flow was found.

Cat

In 1960 Raynor and Ross described how they performed a craniotomy to expose the left hemisphere in the cat (73). Mechanical stroking of the internal carotid/middle cerebral artery led to vasospasm of those vessels as visualized by the camera microscope. Raynor et al. described how they used a similar exposure in 1961 to study the cerebrovascular effects of topically applied serotonin (74).

In 1968 Kapp et al. were the first to report the use of the cat in a subarachnoid hemorrhage model of vasospasm (11,75,76). Using a transclival approach the basilar artery was widely exposed so that it could be visualized with a microscope and direct measurements of its diameter could be made. Various substances, including blood, were placed around the artery (76). Vasoconstriction was observed up to 90 minutes after exposure. This model and slight modifications of it have been used in subsequent studies of vasospasm (77-82).

In 1971 Yamaguchi and Waltz described how they exposed the right middle cerebral artery in the cat via a craniotomy (83). The middle cerebral artery was then punctured with the tip if a No. 11 blade to cause a SAH. The cerebral blood flow was observed to be diminished up to 2 hours after the induction of SAH which the authors attributed to vasospasm.

In 1977 Hayakawa and Waltz compared three techniques to produce a SAH in cats, "puncture device", "tearing device", and "withdrawal puncture device", which were modifications of each other in which a small needle was used to pierce the left middle cerebral artery (84). In all techniques the contents of the left orbit were removed, the optic foramen was enlarged, and with the aid of an operating microscope the proximal part of the left middle cerebral artery was visualized. In these experiments biphasic

increases of epidural pressure were correlated with the extent and distribution of SAH. However, vessel calibre was not determined.

In 1978 Mayberg et al. described the injection of autologous blood via a femoral catheter into the cisterna magna of cats, percutaneously punctured with a small gauge needle (85). Angiographic vasospasm was present 3 days post-SAH and had diminished by 7 days post-SAH. This method has been used in subsequent studies of vasospasm (86,87).

In 1982 Trojanowski described a subarachnoid needle was placed under the zygomatic arch into the prechiasmatic cistern of cats (88). Blood under arterial pressure was introduced into the subarachnoid space by connecting a femoral arterial catheter directly to the needle. In a subsequent study, using the same model, Trojanowski demonstrated that this method resulted in a cerebral blood flow that was diminished 1 hour after SAH induction (89).

Pig

In 1984 Takemae et al. were the first to report the use of the pig in an experimental model of vasospasm (90). Via a C-2 laminectomy a catheter was inserted into the prepontine cistern using microsurgical technique. Blood was injected through the catheter between 5 and 8 days after the laminectomy, and then again 2 days later. Angiographic vasospasm was observed in most of the pigs 2 days after the second blood injection.

In 1990 Mayberg et al. described how they used microsurgical technique to perform a left frontotemporal craniotomy in pigs (91,92). The arachnoid over the left internal carotid artery and the proximal segment of the middle cerebral artery was opened and autologous blood was directly applied to the middle cerebral artery and held in place with a silastic cup sealed with petroleum. After 10 days of exposure to blood the middle cerebral arteries showed luminal narrowing by direct measurement as well as ultrastructural changes affecting all layers of the vessel wall.

Dog

In 1928 Bagley was the first to report the injection of blood into the subarachnoid space of an animal (93). The purpose was to experimentally produce lesions simulating those occurring in humans when a small amount of blood escapes into the subarachnoid space. Of course there was no specific reference to the study of vasospasm. Bagley injected whole blood obtained from the vein of the dog's leg into the cisterna magna, the subarachnoid space over the cerebral hemispheres and occasionally into the cerebral ventricles. Dogs injected with blood exhibited an altered clinical course and there were pathological changes in stained sections of the dogs' brains.

In 1961 Lougheed and Tom were the first to report the use of the dog in an experimental model of subarachnoid hemorrhage with one of the goals to study vasospasm (94). A transoral approach was used to expose the chiasmatic cistern into which autologous blood was injected using a small gauge needle. Animals were sacrificed at 24, 48, 96 hours, one week, and two to three months postoperatively, with tissues undergoing gross and microscopic analysis. Some of the animals became symptomatic after SAH. No comment was made on the presence of vascular constriction.

In 1962 McQueen and Jeanes introduced another method of injecting blood into the chiasmatic cistern using a needle passed under the zygomatic arch and directed towards the optic foramen (95). However the purpose of this study, and subsequent ones using the same model (96,97), was to look at increased intracranial pressure rather than vasospasm. The technique of McQueen and Jeanes has been used in subsequent studies of vasospasm (98).

In 1962 Bradford and Sharkey described how they injected blood into the subarachnoid space via a catheter introduced through a lumbar laminectomy (99). The purpose of this study was to determine the physiologic effects of blood and other test substances in the subarachnoid space and the presence of vasospasm was not determined.

In 1966 Allcock reported on the injection of chemical vasoconstrictors such as serotonin intravascularly into the carotid artery of dogs and tried to correlate the resulting angiographic vessel narrowing with that observed on angiograms of patients in vasospasm

after subarachnoid hemorrhage (100). However no subarachnoid blood was introduced into the dog in this study.

In 1967 Brawley et al. described the exposure of the intracranial internal carotid artery via a subtemporal craniectomy (101). In order to determine the size of the vessel a mercury-in-silastic strain gauge was positioned around the vessel (102). A 4-0 silk ligature was tied loosely around the anterior cerebral artery and the suture brought out through the skin of the external auditory canal. A SAH was created when the suture was pulled, rupturing the anterior cerebral artery. Using this technique the authors observed that cerebral vasospasm was a biphasic process: the acute phase occurred within minutes of rupture and lasted less than 1 hour while the chronic phase occurred 3 to 24 hours after the hemorrhage and lasted for several days.

In 1970 Arutiunov et al. reported on a series of experiments in which SAH was induced by cisternal injection of blood and by injuring vessels in the cisterna magna using a needle (103). The exact technique by which these different methods were performed was not described. Vasospasm was documented by pathological examination 2 to 3 hours after SAH induction.

In 1970 Wilkins and Levitt described several methods of inducing SAH in dogs (104). These included direct injection of autologous blood into the cisterna magna and exposure of the intracranial internal carotid artery. They observed that cisternal injection resulted in limited subarachnoid hemorrhage and no recognizable vasospasm. They determined that the methods of Lougheed and Tom (94) or of McQueen and Jeanes (95) produced significant arterial spasm.

In 1972 Kuwayama et al. described how they injected autologous blood into the cisterna magna of beagle dogs using a small gauge needle (105,106). In order to facilitate the contact of blood with the basilar artery the dog was tilted tail-up at 30 degrees for ten minutes following the injection. Acute angiographic vasospasm (29% to 59% of the original vessel diameter) was observed within the first 120 minutes after injection and chronic angiographic vasospasm (25% to 59% of the original vessel diameter) was observed between 2 and 14 days after injection (105). This model or modifications of it have been used in subsequent studies of vasospasm (4,17,107-121).

In 1973 Jakubowski et al. described a procedure in which blood was injected into the chiasmatic cistern via the foramen opticum using a transorbital route (122). Angiographic vasospasm was present up to 4.5 hours after SAH induction. This model or modifications of it have been used in subsequent studies of vasospasm (123,124).

In 1974 Nagai et al. reported a technique in which a left subtemporal craniotomy was used to expose the posterior communicating artery in the dog (125). The vessel was punctured with a threaded needle which was left in place and the dura tightly closed with the thread externalized through the external auditory canal. Two to three days latter rupture of the artery was produced by pulling out the suture. Using this method the authors demonstrated biphasic angiographic vasospasm: the early spasm lasted 60 minutes and the late spasm began 3 or 4 hours after subarachnoid hemorrhage and continued for several days. This model or modifications of it have been used for subsequent studies of SAH and vasospasm (126,127)

In 1975 Steiner et al. described how they produced subarachnoid hemorrhages in dogs using an extracorporeal shunt, by letting arterial blood pass from one femoral artery through an electronic drop recorder into one of the following sites: chiasmatic cistern, cisterna magna and the lumbar subarachnoid space (128). Since the purpose of the experiments was to determine the effects of repeated hemorrhages on intracranial pressure and other parameters, vasospasm was not studied. This model in a slightly modified form has been used for subsequent studies on SAH and vasospasm (129).

In 1977 Estanol et al. described how they made burr-holes in the parieto-occipital region of the dog and passed a small catheter into the subarachnoid space (130). This catheter was attached to a three way stopcock and then inserted into a peripheral artery. SAH was created by opening the stopcock to allow blood to flow into the subarachnoid space. Since the purpose of the experiments was to determine the electrocardiographic effects of SAH, vasospasm was not studied.

In 1983 Varsos et al. reported on a significant modification of their own model of vasospasm (131). Using small gauge needles they performed two injections of autologous blood into the cisterna magna of the dog 48 hours apart. This model produced significant angiographic vasospasm that was refractory to all of the

pharmacological vasodilators that they tested. They felt that this "two-hemorrhage" model of vasospasm was a better simulation of the vasospasm observed in humans than was their own "one-hemorrhage" model of vasospasm (105) (see above). This model or slight modifications have been used extensively in subsequent studies on SAH and vasospasm (17,67,116,118-120,132-152) and it is probably the most frequently used animal model in the field of vasospasm research.

In 1986 Zabramski et al. introduced a "three-hemorrhage" model of vasospasm by performing three injections of blood into the cisterna magna using a small gauge needle (153). One injection was performed every eight overs over a 24 hour period. Significant angiographic vasospasm was obtained 7 days after SAH induction. This model has been used in subsequent studies of SAH and vasospasm (154).

Primate

In 1965 Echlin was the first to report the use of primates in an experimental model of SAH and vasospasm (155). Using a transoral approach the base of the monkey skull was exposed. This allowed exposure of the basilar and vertebral arteries. Test substances including autologous blood were applied to the vessels and photographs taken to document changes in vessel caliber. The author observed that the vessels exhibited marked constriction when bathed in subarachnoid blood and that vasospasm could be general or local depending on whether a localized or widespread area of the vessel was in contact with the blood. This model or modifications of it have been used in subsequent studies of SAH and vasospasm (156).

In 1968 Simeone et al. described how they performed a small anterior craniectomy at the lateral margin of the sphenoid wing in the rhesus monkey (157). The intracranial internal carotid artery at its bifurcation into the anterior and middle cerebral arteries was exposed and a small catheter left in the subarachnoid space adjacent to them. This catheter was externalized through the craniotomy and at a later date by means of a small gauge needle one of the intracranial vessels could be punctured to produce a SAH. This technique produced immediate and durable angiographic vasospasm which persisted in some experiments for up to 4 days post-SAH. This model or modifications of it have

been used in subsequent studies of SAH and vasospasm (158-160).

In 1968 Chow et al. reported on the use of cynomologous monkeys to test two techniques of SAH induction (161). In the first method autologous blood was injected directly into the cisterna magna using a small gauge needle. In the second method a low cervical laminectomy was performed and a small catheter threaded into the cisterna magna through which blood could be instilled. Both methods produced angiographic vasospasm that persisted for up to 3 hours after SAH induction. Both of these methods or modifications of them have been used in subsequent studies of SAH and vasospasm: direct cisternal injection (159,162-167) and catheter into cisterna magna (168).

In 1968 Landau and Ranshoff described a series of experiments in African green monkeys designed to compare the degree of vasospasm produced by puncturing an intracranial vessel with that produced by placement of blood around an intracranial vessel (168). In one study group a frontotemporal craniotomy was performed with exposure of the supraclinoid internal carotid artery. The vessel was punctured with a small needle. In another study group a small silastic catheter was placed into the cisterna magna through a high cervical laminectomy and autologous blood injected. The authors observed that although angiographic spasm was seen in both groups, the spasm was more consistent, and severe, and of greater duration in the vessel-puncture group, and the phenomenon of late spasm was more prominent in this group as well (up to 20 days).

In 1970 Weir et al. described how they made a burr-hole in the midline of the rhesus monkey skull just cephalad to the nasion (169). This allowed the passage of a small gauge needle through the skull in the midline under the frontal lobes along the floor of the anterior fossa. Under fluoroscopic control the needle was positioned just anterior to the tuberculum sella, in the subarachnoid space dorsal to the planum sphenoidale. The authors performed repeated injections of autologous blood through this needle at weekly intervals. Angiographic vasospasm was limited to the intradural cerebral vessels and was diffuse. It never lasted longer than a few hours and late vasospasm did not occur. This model or modifications of it have been used for subsequent studies on SAH and vasospasm (170-176).

In 1971 Echlin described how the dura over the anterior spinal cord was exposed in the monkey via an anterior approach through the bodies of the cervical vertebrae C-3 to D-2 (177). A catheter was placed into the subarachnoid space in the midline through which autologous blood could be injected. This technique consistently produced acute and chronic angiographic vasospasm that lasted for up to 14 days.

In 1972 Simeone et al. reported on another set of experiments designed to compare the techniques of vessel puncture and blood instillation (178). In rhesus monkey a small craniectomy was made to allow exposure of the intracranial internal carotid artery. In one group of animals a small catheter was left in place and autologous blood injected into the subarachnoid space around the internal carotid artery. In a second group of animals the internal carotid artery was punctured with a small gauge needle. The authors found that the puncture technique produced biphasic spasm that lasted longer than the instillation technique.

In 1972 Hashi et al. described a technique in which a SAH was produced in baboons by injecting autologous blood through a catheter inserted transorbitally into the prechiasmatic cistern (179). Angiographic vasospasm was observed up to 48 hours after SAH induction.

In 1976 Alksne and Branson described how they performed a C2 laminectomy in a monkey and passed a small catheter ventral to the spinal cord into the prepontine cistern through which autologous blood could be injected (180). Seventeen days after SAH induction vessels in the Circle of Willis exhibited pathological changes that were felt to be consistent with vasospasm.

In 1979 Boisvert et al. described how they passed a small gauge needle percutaneously through the optic foramen into the suprachiasmatic cistern in baboons (181). Although only a few animals underwent angiography, no vasospasm was evident one week after SAH induction. This model or modifications of it have been used on subsequent studies of SAH (182,183).

In 1981 Clower et al. reported on yet another study to compare the techniques of blood instillation and vessel puncture (184). In one group of rhesus monkeys autologous blood was injected into the subarachnoid space via the optic foramen. In a second group

of animals after intraorbital exenteration a small craniotomy was performed adjacent to the optic foramen exposing the middle cerebral artery proximal to the lateral fissure. A 7-0 suture was passed through the vessel and externalized. After 7 days the suture was pulled out resulting in a SAH. Animals in both groups were sacrificed at intervals for up to 30 days. Vessels surrounded by subarachnoid blood after vessel rupture exhibited histological changes that were more consistent with vasospasm than vessels that had been subjected to instilled blood.

In 1982 Jakubowski et al. described how an established transorbital approach (185) was used to expose the right posterior communicating artery (186). A loop of 5-0 nylon was passed around the vessel and a small gauge needle passed over nylon so that the needle was adjacent to the vessel wall. The nylon/needle was externalized so that the nylon snare could be pulled at a latter date in order to cause a SAH by transecting the posterior communicating artery. Since the purpose of the experiments was to study cerebral blood flow after SAH the presence of vasospasm was not determined. This model or modifications of it have been used for subsequent studies of SAH and vasospasm (187).

In 1982 Peerless et al. introduced a customized experimental stand to hold macaque monkeys in a prone position (188). With the head positioned so that the orbito-meatal line was vertical a small gauge needle was passed into the basal cisterns and autologous blood injected. Angiographic vasospasm was observed 1 hour and 1 and 2 weeks after SAH induction.

In 1982 Frazee described how a fronto-temporal craniectomy was performed in monkeys in order to expose the intracranial carotid artery at the origin of the posterior communicating artery (189). A small gauge needle was placed through both walls of the carotid artery and attached to a suture that was externalized. The following day the suture was pulled, removing the needle and causing a SAH. Angiographic vasospasm appeared 4 days after SAH induction and persisted for at least 11 days. This model or modifications of it have been used in subsequent models of SAH and vasospasm (190,191).

In 1982 Espinosa et al. described how a small gauge needle was positioned under fluoroscopic monitoring through a midline frontal twist drillhole along the floor of the anterior fossa into the chiasmatic cistern (192). This needle was secured to the skull with a screw device and used to instill autologous blood into the subarachnoid space at various intervals. Computerized tomography scanning and cerebral angiography were performed on animals and the degree of vasospasm was found to correlate with the amount of blood in the subarachnoid space. Vasospasm was significantly higher on days 0, 7, and 14 than at other times.

In 1984 Espinosa et al. reported how the operating microscope could be used to perform a frontal craniectomy in the cynomolgus monkey, opening the arachnoid cisterns over the internal carotid artery, the posterior communicating artery, the middle cerebral artery, and the anterior cerebral artery (193). After removal of 3 to 4 ml of cerebrospinal fluid 7 ml of autologous blood was placed over the exposed arteries and the dura closed in a watertight fashion. There was significant angiographic vasospasm (31% to 100% reduction in vessel calibre) 7 and 14 days post-SAH induction. This model or modifications of it have been used in subsequent studies of SAH and vasospasm (194-206).

In 1992 Delgado-Zygmunt et al. described how they implanted a small catheter into the cisterna magna of a squirrel monkey through which autologous blood could be injected (207). In addition a small cannula was inserted sterotactically into the interpeduncular cistern through which additional autologous blood could be injected. Angiography of the vertebro-basilar and right internal carotid arteries revealed a biphasic vasospasm with a maximal acute spasm at 10 minutes and maximal late spasm at 6 days after blood injection. This model has been used in subsequent studies of SAH and vasospasm (208).

Summary and Discussion

In the models described above 28% use a technique in which the vessel is punctured or torn to induce a SAH (and vasospasm) while 72% use a technique in which blood is placed or injected around blood vessels to induce vasospasm. In humans

vasospasm usually occurs following a SAH secondary to aneurysmal rupture, a process in which blood escapes under relatively high pressure from large intracranial arteries at the base of the brain. There is evidence that the degree of vasospasm which develops is related to the volume and location of the blood in the subarachnoid cisterns (209,210). However, it is not clear how important the arterial rupture and the immediate pressure effects of the ejected blood are to vasospasm development. Obviously those groups who use puncture or tearing techniques feel that it is important to try and simulate this aspect of the process, while those who place or inject blood around arteries feel that it is the actual presence of large volumes of blood in contact with the vessel (irrespective of how it got there) that is the overriding factor in vasospasm development. The three studies that attempted to compare the two techniques were all performed in primates (168,178,184) and all concluded that the vessel puncture technique was superior in the degree and the morphological appearance of subsequent vasospasm. Nevertheless, as shown in numerous models, placement or injection of blood around arteries in the basal cisterns still results in consistent and significant vasospasm. Moreover, these techniques are generally easier to perform and are associated with less animal morbidity and mortality, explaining the greater popularity of models that employ them.

It is interesting that so many different *in vivo* animal models of vasospasm have been developed in so many different species. *In vivo* experimental models have been used to investigate diverse aspects of vasospasm including its natural history (63,211), pathogenesis (92,132,202,212), pathology (91,184,197,200), diagnosis (63,100), and treatment (12,57,58,106,119,143,146,176,193,194,213,214). Hence a model used to investigate one aspect may not be suitable for another. Also, some models require specialized equipment, facilities, and personnel which may not be available in all laboratories. Cost may also be a factor since some models, especially those involving primates (192,193), are quite expensive. Clearly no one model meets all the criteria as laid out by previous authors (14,15) or in the introduction to this review. Some of the models are probably unnecessarily complex (46,84,101) or more importantly don't consistently and reproducibly produce vasospasm (90,181); most of these models were used only once or twice and then abandoned.

There is a relative cost advantage to using rats and rabbits and therefore large numbers of animals are more readily used in studies involving these models. However, the small size of the vessels in these species makes it harder to perform angiography and angioplasty and it requires a greater leap to apply results from these models to human vasospasm. These models may be ideal to initially screen new pharmacological therapies in large numbers of animals before moving to more expensive large animal models (12,55,59,60,62).

Cat, pig and dog models of SAH and vasospasm are generally more costly than rat or rabbit models. However the cerebral vessels in these species are larger, more easily subject to angiography and angioplasty, and the results of experiments more readily extrapolated to human vasospasm (13,91,92,145). If the number of studies using a certain model is any sign of its merit, then the canine model of Varsos et al. (131) has gained widest acceptance in vasospasm research. This model, which involves a double injection of blood into the dog's basal cistern, is the most popular vasospasm model and its use facilitates comparison between studies at different centers.

Since primates are phylogenetically closest to humans it may be that models using primates provide results that are most applicable to vasospasm in humans. The primate model of Espinosa et al. (193), developed in the laboratory of Bryce Weir in Edmonton, Alberta, has been particularly useful in this regard and is most likely the best model of SAH and vasospasm available today. Many of the most significant contributions to the field of vasospasm research have been made using this model, including observations on the pathological changes that occur in vasospasm (200,215), the determination that hemoglobin is the principle spasmogen in vasospasm (202), that surgical or fibrinolytic clot removal will decrease vasospasm (198,213,216), and that nimodipine and tirilazad mesylate may be efficacious in the treatment of vasospasm (195,214,217-219). A number of new and promising therapies are also being investigated using this model (206,220-223).

In Vivo Models of SAH and Vasospasm Using Extracranial Arteries

A number of *in vivo* animal models of vasospasm that use extracranial arteries have now been developed. The advantages of these models include: (1) the application and maintenance of blood clot around the arteries is technically less demanding; (2) the arteries are more accessible for endovascular procedures such as transluminal balloon angioplasty; and (3) operative morbidity and mortality is lower, animal handling is simpler, and overall cost of experiments are lower especially when small animal models are used. The principle disadvantage is that the arteries under study are non-cerebral. Cerebral arteries differ from their systemic counterparts in several ways, including endothelial permeability, the nature of the adventitial matrix, and the manner in which they respond to certain vasoactive agents (224-234). The importance of these differences as they pertain to the actual pathogenesis and treatment of vasospasm following periarterial blood clot placement has yet to be fully ascertained. There is evidence that smooth muscle vascular spasm is a general phenomenon (211,235-238). It most likely becomes a clinical problem following aneurysmal subarachnoid hemorrhage because (1) large volumes of blood remain entrapped in the basal cisterns around intracranial vessels (and such circumferential and thick periarterial clots are unlikely to form around systemic arteries travelling in soft tissues outside of the skull), (2) there are fewer mechanisms in the cerebrospinal fluid to dissipate and phagocytize blood breakdown products than there are in the soft tissues around periarterial vessels, and (3) the brain is a more sensitive organ to even slight oxygen and nutrient deprivation. At present there are three models of vasospasm that use extracranial arteries.

In 1984 Pickard et al. were the first to report on the use of extracranial arteries specifically to study periarterial blood clot induced vasospasm as it related to the phenomenon of cerebral vasospasm (239). In rabbits both common carotid arteries were exposed and autologous blood was placed around one carotid artery and held in place with a polyvinyl chloride cuff; the other artery acted as a control. Animals were sacrificed 7 days later and both arteries removed for study. Angiographic or morphologic arterial narrowing was not studied in these experiments. However blood coated arteries exhibited increased eicosanoid production compared to control arteries.

In 1990 Okada et al., reported on a model in which they exposed segments of both proximal femoral arteries in the inguinal region of rats using microsurgical technique (235). Autologous blood was applied directly to one of the femoral arteries and the artery then covered with a silastic cuff to contain the clot. As compared with matched control arteries, there was a reduction in luminal cross-sectional area 7 days later in vessels exposed to blood and the vessels demonstrated morphological changes similar to those seen in cerebral vasospasm after SAH. The authors concluded that the chronic narrowing in the rat femoral artery exposed to periadventitial blood was analogous to that observed in cerebral arterial vasospasm after SAH and that this model represented a simple and reliable means to investigate pathogenic mechanisms and potential therapies for vasospasm. The model or slight modifications of it have been used for subsequent studies of SAH and vasospasm (240,241). It should be noted that in the original paper Okada et al. reported unpublished results indicating that whole blood in silastic cuffs applied to rat carotid arteries (which have thicker adventitia than rat femoral arteries) produced vasospasm only when the adventitia was extensively removed from the artery (235).

In 1991 Macfarlane et al. described how they exposed both common carotid arteries in rabbits through a midline cervical incision and excised all loose areolar tissue investing the vessels (236). Blood filled silicone tubing was placed around both carotid arteries. Angiographic vasospasm was observed within 24 hours, reached a maximum between 24 and 48 hours (53% of control diameter), began to resolve by 72 hours and was almost completely absent 6 days after the initial blood application. Arteries in vasospasm exhibited characteristic morphological changes. This model or slight modifications of it have been used for subsequent studies of vasospasm (237,238).

A New Model of Vasospasm Using the Canine High Cervical Internal Carotid Artery

The results presented in this report support the use of the canine high cervical internal artery for the study of vasospasm caused by the presence of periarterial blood. In this study the placement of an empty silicone elastomer cuff around the canine ICA did not cause angiographic vasoconstriction or measurable arterial luminal narrowing

after 7 days. Pharmacological responsiveness of these arteries was not different than that observed in normal control arteries. Morphological features of these arteries as determined by electron microscopy were also the same as normal control arteries.

Placement of a silicone elastomer cuff filled with blood around the canine ICA produced consistent and reproducible angiographic vasoconstriction and measurable arterial narrowing after 7 days (vasospasm). Pharmacological responsiveness of the vasospastic arteries was generally diminished when compared to normal control arteries, although this feature may be a variable characteristic of vasospasm as noted in other *in vivo* models (13,238). Morphological features of vasospastic arteries as determined by electron microscopy were consistent with those observed using other models (13,200,235,237).

Further characterization of the time course of vasospasm in this model is required. Nevertheless, this model should prove useful in future experiments on vasospasm since the vessels under study are of the same caliber as the larger cerebral arteries at the base of the human brain and in particular are suitable for endovascular manipulation with the same balloon catheters used in the treatment of vasospasm in humans.

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Table II-1

Selected *in vivo* animal models of subarachnoid hemorrhage (SAH) and vasospasm

In vivo models of SAH and vasospasm using intracranial arteries

Species	Year	Technique	Ref.
Rat	1979	basilar artery puncture	18
Rat	1985	blood through catheter to cisterna magna	26
Rabbit	1984	blood injected into cisterna magna x 1	47
Cat	1968	basilar artery exposed; blood added	75
Pig	1990	internal carotid artery exposed; blood added	91
Dog	1961	chiasmatic cistern exposed; blood added	94
Dog	1972	blood injected into cisterna magna x 1	105
Dog	1983	blood injected into cisterna magna x 2 "the canine double hemorrhage model"	131
Dog	1986	blood injected into cisterna magna x 3	153
Primate	1965	basilar artery exposed; blood added	155
Primate	1968	craniectomy; basal arteries punctured	157
Primate	1970	needle to anterior fossa; blood injected	169
Primate	1984	craniotomy; basal arteries exposed; blood added "the Edmonton model" or "the Weir model"	193

In vivo models of SAH and vasospasm using extracranial arteries

Rabbit	1984	blood around common carotid artery	239
Rat	1990	blood around femoral artery	235
Rabbit	1991	blood around common carotid artery	236

Table II-2

Features of *in vivo* animal models of subarachnoid hemorrhage (SAH) and vasospasm

<u>*In vivo* models of SAH and vasospasm using intracranial arteries</u>

Rabbit and rat models

Principal Advantages:	1. Lower cost of experiments. 2. Procedures easier to perform.
Principal Disadvantages:	1. Species phylogenetically farthest from humans. 2. Harder to angiographically quantify vasospasm in small vessels.

Dog, cat, and pig models

Principal Advantages:	1. Species phylogenetically closer to humans. 2. Easier to angiographically quantify vasospasm in larger vessels.
Principal Disadvantages:	1. Higher cost of experiments. 2. Procedures more difficult to perform.

Primate models

Principal Advantages:	1. Species phylogenetically closest to humans. 2. Easier to angiographically quantify vasospasm in larger vessels.
Principal Disadvantages:	1. Very high cost of experiments. 2. Procedures usually the most difficult to perform.

<u>*In vivo* models of SAH and vasospasm using extracranial arteries</u>

Principal Advantages:	1. Lower cost of experiments. 2. Procedures easier to perform. 3. Endovascular techniques such as transluminal balloon angioplasty are easier to perform.
Principal Disadvantage:	1. Arteries are not intracranial, so how experimental results pertain to human cerebral vasospasm is less certain.

STUDY DESIGN

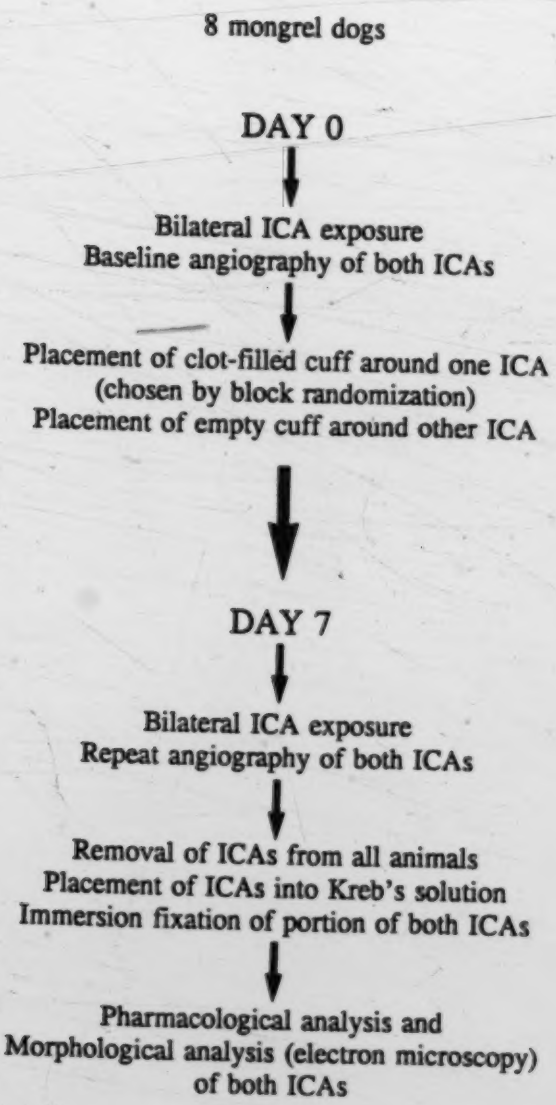
8 mongrel dogs
DAY 0
Bilateral ICA exposure
Baseline angiography of both ICAs
Placement of clot-filled cuff around one ICA
(chosen by block randomization)
Placement of empty cuff around other ICA
DAY 7
Bilateral ICA exposure
Repeat angiography of both ICAs
Removal of ICAs from all animals
Placement of ICAs into Kreb's solution
Immersion fixation of portion of both ICAs
Pharmacological analysis and
Morphological analysis (electron microscopy)
of both ICAs

Figure II-1
Study design for the experiment to determine the effects after 7 days of placing a blood-clot filled cuff and an empty cuff around the canine high cervical carotid artery. ICA=internal carotid artery.

Figure II-2
Operating room set-up used for canine high cervical internal carotid artery model of vasospasm. Dog was positioned supine and breathed room air spontaneously through an endotracheal tube. The anterior aspect of the neck was prepped and draped using aseptic technique. The operating microscope was available for use throughout the procedure. Angiography equipment is shown in the background.

Figure II-3
Intraoperative photograph shows divided anterior neck muscles and trachea exposed in the midline.

Figure II-4
Intraoperative photograph shows high cervical internal carotid artery exposed on the left side. A similar exposure was performed on the right side.

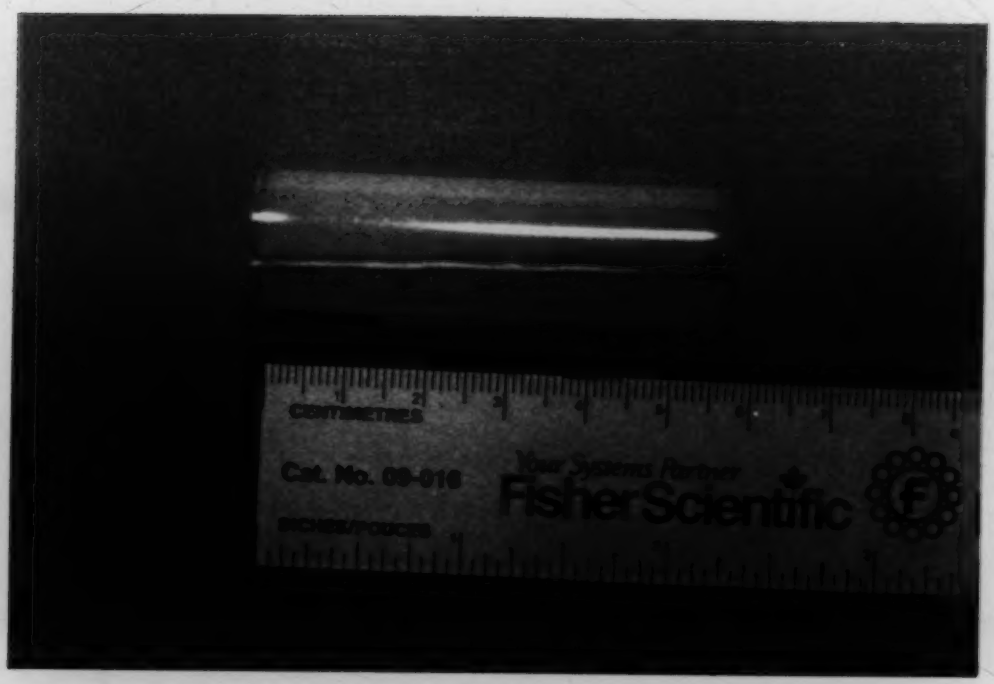

Figure II-5
Silicone elastomer cuff used to surround high cervical internal carotid artery. Cuff was 50 to 60 mm in length and had a 10 mm internal diameter.

Figure II-6
Intraoperative photograph shows silicone elastomer cuff positioned around left high cervical internal carotid artery. Autologous clotted arterial blood is placed inside the cuff and around the artery. Note the small cotton pledgets located at either end.

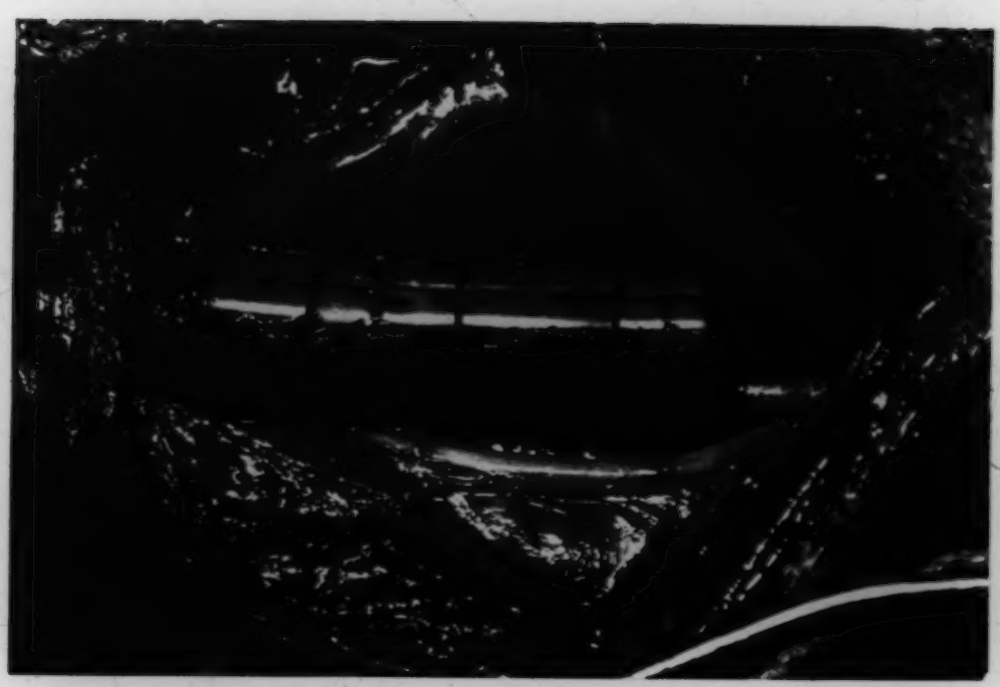

Figure II-7
Intraoperative photograph shows silicone elastomer cuff positioned around left high cervical internal carotid artery and secured with three silk ties along its length. Inside cuff is 10 mL of autologous clotted arterial blood contained by small cotton pledgets located at either end.

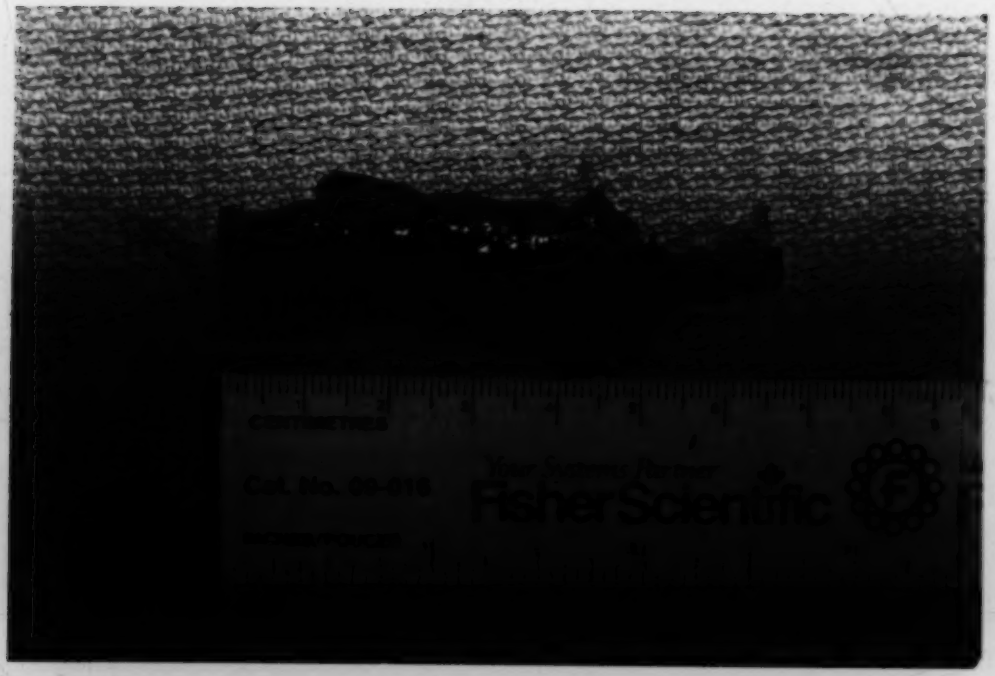

Figure II-8
Left high cervical internal carotid artery after en bloc removal on day 7. Artery was placed immediately into oxygenated Kreb's solution and prepared for pharmacological and morphological analyses.

Figure II-9
Apparatus comprised of organ baths and force-displacement transducers (left) attached to polygraph (right) used for pharmacological studies.

Figure II-10
Representative angiograms of artery surrounded by blood clot-filled silicone elastomer cuff on day 0 (A) and day 7 (B). Note arterial narrowing (vasospasm; beginning at upper arrow and continuing to lower arrow) after arterial segment encased in blood clot-filled silicone elastomer cuff for 7 days.

Figure II-11
Representative angiograms of artery surrounded by an empty silicone elastomer cuff on day 0 (A) and day 7 (B). Note absence of arterial narrowing after arterial segment surrounded by an empty silicone elastomer cuff for 7 days.

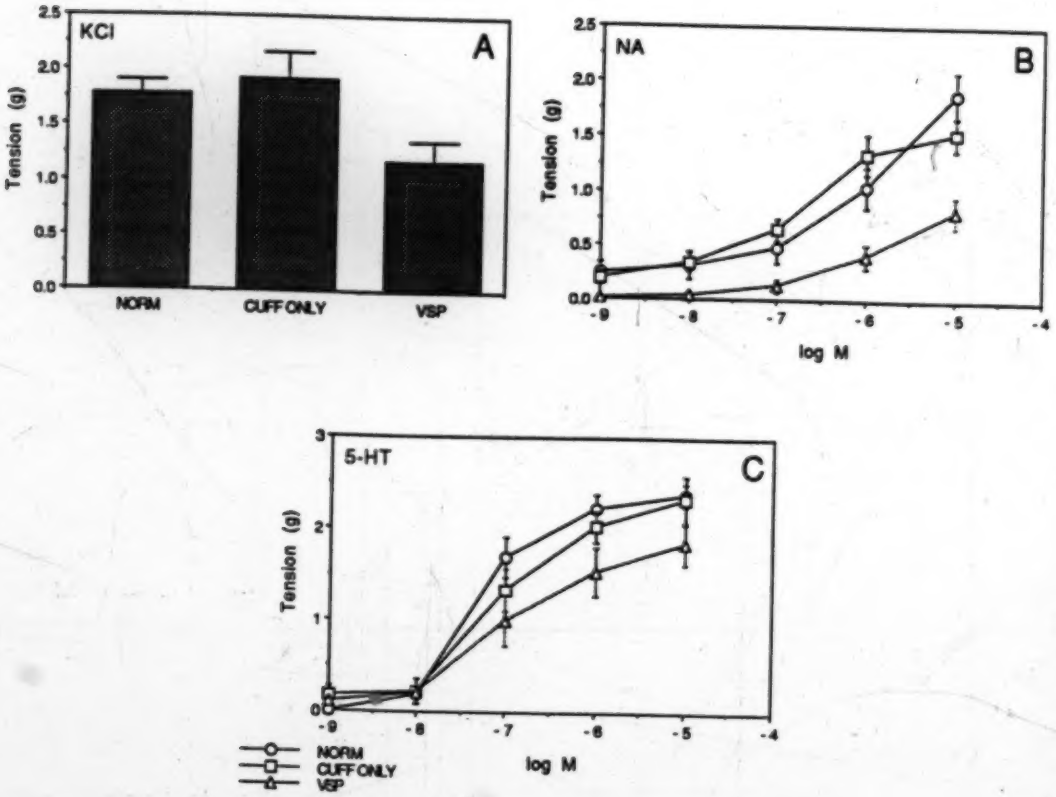

Figure II-12
Concentration-response curves of the three vessel groups (normal control [NORM], cuffed non-blood coated [CUFF ONLY], and cuffed blood coated [VSP]) to the three vasoconstrictors: potassium chloride (KCl) (A); noradrenaline (NA) (B); serotonin (5-HT) (C). One concentration of KCl (60 mmol/L) was tested. Normal control vessels and cuffed non-blood coated vessels responded similarly to all vasoconstrictors. Cuffed blood coated (vasospastic) vessels had diminished responses to all vasoconstrictors but not at a significant level. M indicates vasoconstrictor concentration (molar).

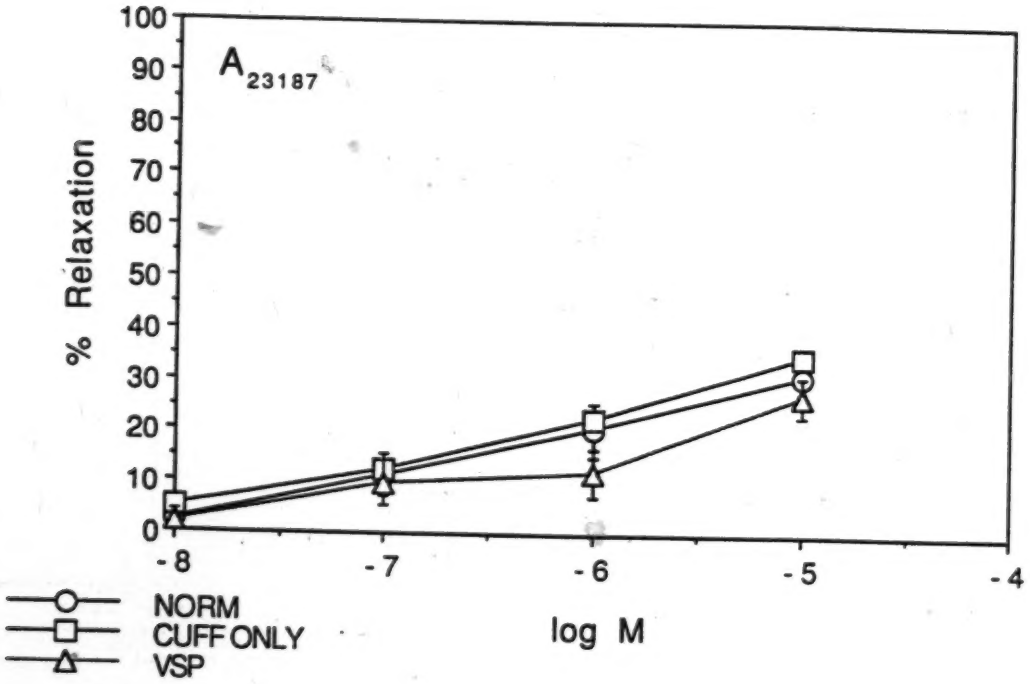

Figure II-13
Concentration-response curves of the three vessel groups (normal control [NORM], cuffed non-blood coated [CUFF ONLY], and cuffed blood coated [VSP]) to the endothelium-dependent vasorelaxant calcium ionophore A_{23187}. Percent relaxation was calculated using tonic contraction with 10^{-5} noradrenaline as 0% relaxation. Normal control vessels and cuffed non-blood coated vessels responded similarly to the calcium ionophore at all concentrations. Cuffed blood coated (vasospastic) vessels had diminished responses to the calcium ionophore but not at a significant level. M indicates vasorelaxant concentration (molar).

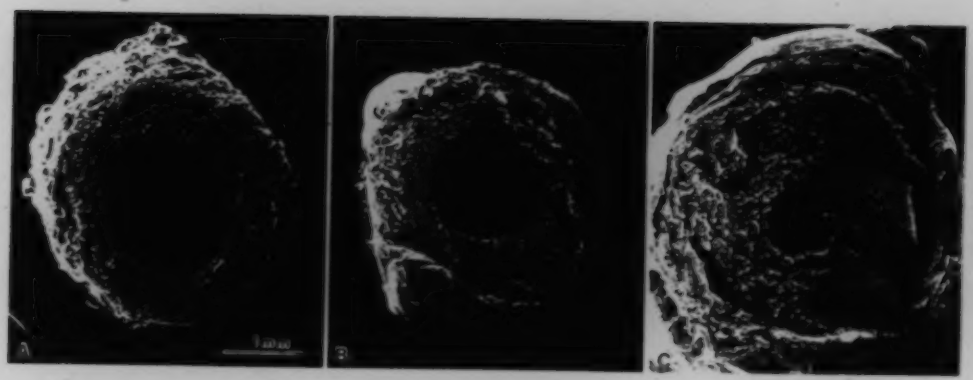

Figure II-14
Scanning electron micrographs show similarities and differences between the three vessel groups. A, Cuffed non-blood coated vessel segment. B, Normal control vessel segment. C, Cuffed blood coated (vasospastic) vessel segment. Note that the presence of an empty cuff around the artery for 7 days does not alter the appearence of the vessel wall (A compared to B): lumen diameter is not decreased, luminal/wall diameter thickness ratio is not decreased and there is no alteration in the appearence of the internal elastic lamina or endothelial surface. In contrast, arteries coated in blood for 7 days exhibit features characteristic of vasospasm (C compared to B and A): smaller lumen, decreased luminal/wall diameter thickness ratio, corrugation of the internal elastic lamina and folding of the endothelial surface.

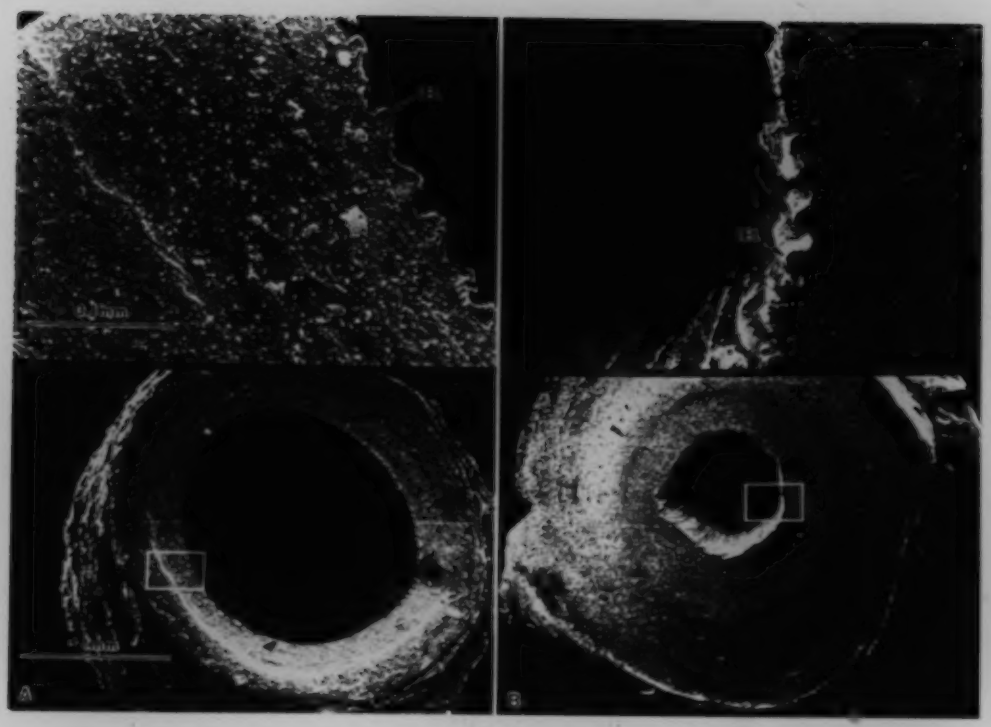

Figure II-15
Scanning electron micrographs, with higher magnification views, highlight differences between normal control vessel segment (A) (upper panel is high power of lower), and cuffed blood coated (vasospastic) vessel segment (B) (upper panel is high power of lower). Vasospastic vessels exhibit a smaller lumen, decreased luminal/wall diameter thickness ratio, corrugation of the internal elastic lamina and folding of the endothelial surface.

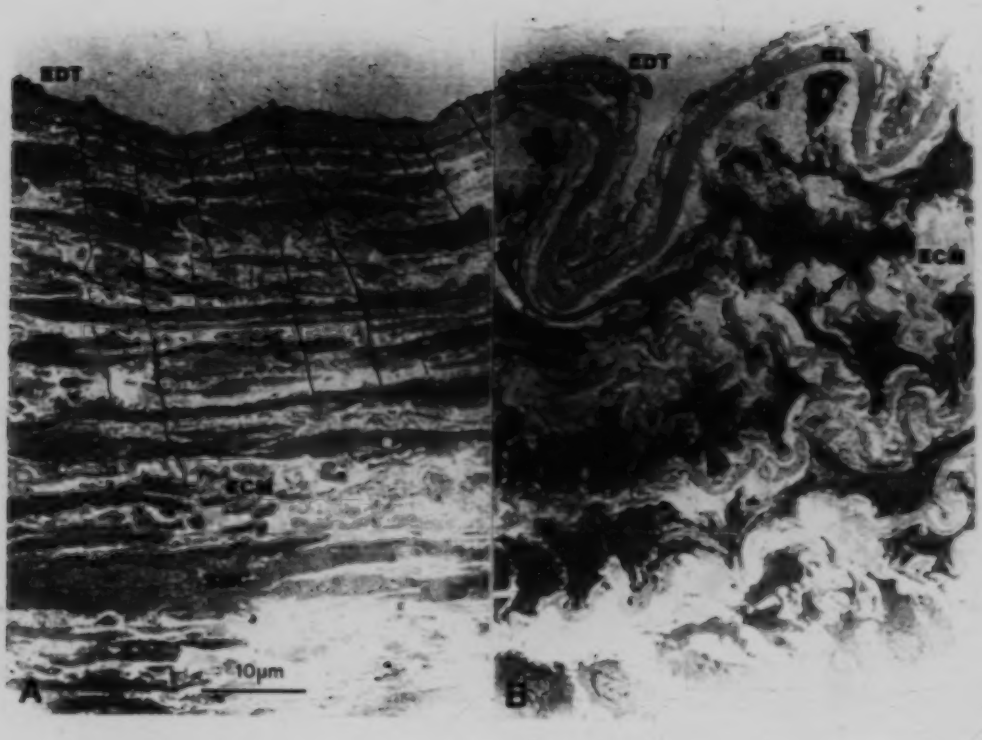

Figure II-16
Transmission electron micrographs show differences between normal control vessel segment (A), and cuffed blood coated (vasospastic) vessel segment (B), as seen in cross section of the vessel wall. Normal control vessel shows endothelial cells (EDT), internal elastic lamina (IEL), smooth muscle cells (SMC), and extracellular matrix (ECM). Cuffed blood coated (vasospastic) vessel shows rounding and folding of endothelial cells (EDT), corrugation of the internal elastic lamina (IEL), and rounding with surface rippling (RP) of smooth muscle cells (SMC) within the tunica media.

CHAPTER THREE
IN VIVO ANGIOPLASTY PREVENTS THE DEVELOPMENT OF VASOSPASM IN CANINE CAROTID ARTERIES: PHARMACOLOGICAL AND MORPHOLOGICAL ANALYSES

INTRODUCTION

The development and maintenance of delayed-onset cerebral vasospasm following aneurysmal subarachnoid hemorrhage (SAH) is believed to represent a combination of sustained smooth muscle constriction and structural change both of which cause narrowing of the arterial lumen diameter. The mechanisms involved have been subjected to intensive experimental investigation (1). Severe vasospasm causing symptomatic cerebral ischemia can now be treated with transluminal balloon angioplasty (TBA), which is the mechanical dilatation of vasospastic arteries by means of inflatable microballoons attached to the tips of percutaneously inserted arterial catheters (2-29). While it has been recognized that TBA results in efficient arterial dilatation, and that improvement in cerebral blood flow leads to clinical recovery in a high percentage of patients, the morphological and functional effects of TBA on the cerebral arterial wall have only recently been reported (30-32).

Previous work in this laboratory has demonstrated that in vitro TBA of both normal and vasospastic canine basilar arteries causes an immediate and profound functional impairment of vascular smooth muscle, as well as providing structural evidence of arterial wall stretching (30). However, determination of longer-term effects of TBA requires an animal model of cerebrovascular spasm in which the affected artery is accessible to consistent and safe in vivo TBA. We have thus developed a model of vasospasm using placement of clotted blood around the distal cervical internal carotid artery (ICA).

While it is clear that TBA can dilate spastic vessels, it is not known whether prior TBA can prevent the development of vasospasm. This question is of more than academic interest; if it is possible to alter the function of normal arteries by stretching, and this then renders them insensitive to agents which would normally produce vasospasm, the

observation would have important implications about the mechanism by which vasospasm develops. It would also have clinical implications, in that if prior TBA can prevent the development of cerebrovascular spasm, it would support early TBA to halt the progression of the condition.

MATERIALS AND METHODS

Twelve mongrel dogs of either sex weighing between 16 and 25 kg were used in this study. The protocol was evaluated and approved by the University of Alberta Animal and Ethics Review Committee and experiments were conducted with strict adherence to the standards of the Canadian Council on Animal Care.

Model of Blood Clot-Induced Vasospasm in the Canine High Cervical Carotid Artery

This model is an adaptation of models used by others in the rat femoral artery (33) and in the rabbit cervical carotid artery (31,32,34). We have previously determined that placement of a blood clot held in place by a silicone elastomer cuff around the dog ICA, results in typical angiographic and structural vasospasm seven days later. Placement of a silicone elastomer cuff alone does not affect the vessel wall. For this study the following surgical protocol was adopted (Figure III-1):

Animals were anesthetized with sodium pentobarbital (0.5 mL/kg) and intubated on Day 0 for angiography, TBA, and blood-clot placement. Adequate anesthesia was maintained by administration of intravenous boluses of pentobarbital (0.05 mL/kg). The animals were allowed to breathe room air spontaneously, and arterial blood gases were determined on the first four animals to confirm that the protocol maintained normocarbia. A midline cervical incision was made and both cervical ICAs were exposed and punctured with a 24 gauge angiocatheter for angiography (performed with 5 mL of iothalamate meglumine, injected at a rate of 0.75 mL/s). Using the operating microscope for magnification, 60 mm sections of both ICAs were then dissected free of adjacent tissues.

One of the ICAs was chosen by block-randomization to undergo TBA, and a silicone elastomer balloon angioplasty catheter was introduced into this ICA lumen via

a small arteriotomy proximal to the area of study. The balloon, which was 7 mm in diameter and 12 mm in length when inflated (303.9 to 506.5 kPa pressure), was used to dilate the ICA twice for 10 seconds, each time to approximately 150% of its original diameter along a length of 50 mm. Angiography was then repeated on this artery. Both ICAs were then covered along their dissected segments with 10 mL of autologous arterial clotted blood which was held in place within a 50 mm segment of silicone elastomer tubing cuff with a 10 mm inner diameter. The silicone elastomer tubing was secured with three silk ties along its length. Small cotton pledgets positioned at either end of the tube contained the clot.

The animals were cared for in the usual fashion for 7 days with daily neurological monitoring. There was no mortality or severe morbidity related to the procedures outlined above, and no animal developed a neurological deficit. On Day 7 the animals were again anesthetized, the cervical incision was reopened and angiography was repeated on both ICAs. The animals were then sacrificed with sodium pentobarbital (30 mg/kg).

Four animals, designated as comprising group A, underwent immediate *in situ* perfusion fixation of both ICAs using 2.5% glutaraldehyde in 0.12 mol/L Millonig's buffer solution (pH 7.2). In the remaining group of 8 animals (group B), both ICAs were immediately removed and placed in Krebs solution of the following composition: 120 mmol/L NaCl, 5 mmol/L KCl, 1.5 mmol/L $CaCl_2$, 1 mmol/L KH_2PO_4, 1 mmol/L $MgSO_4$, 25 mmol/L $NaHCO_3$, 55 mmol/L dextrose, aerated with 95% O_2 / 5% CO_2 and maintained at 37°C. These arterial segments were used for pharmacological studies. In addition, a segment of ICA proximal to the original surgical field and the previously dissected ICA segment, was removed on one side from each animal, to provide a control preparation for both the morphological and pharmacological studies. Two rings were cut from each isolated ICA obtained from animals in group B, thus enabling us to examine the pharmacological properties of dilated and nondilated blood-coated ICAs, and normal control ICAs. The diameter of the segments were measured with a micrometer under magnification prior to pharmacological analysis. The remainder of the arterial segments were prepared for morphological analysis after immersion fixation in 2.5%

glutaraldehyde in 0.12 mol/L Millonig's buffer solution (pH 7.2).

Angiographic Measurement of Vasospasm and the Effects of TBA

For all angiograms the diameter of the carotid artery in millimeters was determined by direct measurement of the angiogram, at the point corresponding to the midpoint of the blood-filled silicone elastomer cuff. For the nondilated group, the degree of angiographic vasospasm was determined by comparing vessel diameters on Day 0 and on Day 7 and calculating a percentage change in vessel diameter. For the dilated group, the initial magnitude of the TBA-induced dilatation was estimated by determining the percentage change in vessel diameter on day 0, before and after angioplasty. After seven days, the persistence of the dilatation induced on day 0 was estimated by calculating the percentage change in vessel diameter between that seen on the day 7 angiogram and that seen on the day 0 preangioplasty angiogram.

Pharmacological Studies

Responses of arterial rings were recorded isometrically using force-displacement transducers connected to a polygraph. Rings of cerebral arteries were suspended between two stainless-steel hooks, under a resting tension of 1 g in organ baths of 10 mL working volume containing Krebs bicarbonate solution maintained at 37°C and bubbled with 95% O_2 / 5% CO_2. After an equilibration period of 1 hour, during which the Krebs solution was changed every 15 minutes, the response to potassium chloride (KCl, 60 mmol/L) was recorded and preparations were washed until resting tension was again obtained. Cumulative dose-response curves for noradrenaline (10^{-9} to 10^{-5} mol/L), serotonin (5-HT, 10^{-9} to 10^{-5} mol/L), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10^{-9} to 10^{-6} mol/L) were then recorded for each arterial ring. The preparations were preconstricted with noradrenaline (10^{-5} mol/L) prior to vasorelaxation studies in which cumulative dose-response curves to the calcium ionophore A_{23187} (10^{-8} to 10^{-5} mol/L) and to bradykinin (10^{-12} to 10^{-7} mol/L) were obtained. The response to papaverine (5×10^{-4} mol/L) was also recorded after tonic contraction using noradrenaline (10^{-5} mol/L) had been established. Preparations were washed until the resting tension had been restored, before another agent was tested. The

ring preparations were studied in the organ baths for approximately 8 to 10 hours. At the end of each experiment the ring preparations were tested with KCl (60 mmol/L) to confirm that the responses were not different from the initial responses obtained to KCl at the beginning of the experiment.

Electron Microscopy

Segments from the three groups of vessels were examined with transmission electron microscopy and scanning electron microscopy. Cross-sections of the vessel wall were examined with transmission electron microscopy. The intact vessel wall (luminal and cross-sectional aspects) was examined with scanning electron microscopy.

All specimens were prefixed in 2.5% glutaraldehyde in 0.12 mol/L Millonig's buffer solution (pH 7.2) overnight at room temperature. After samples had been washed three times for 15 minutes each in Millonig's buffer, they were postfixed with 1% osmium tetroxide (OsO_4) in the same buffer for 2 hours. These samples were briefly washed in distilled water, and dehydrated in a graded series of ethanol solutions (50%, 60%, 70%, 80%, and 90%; 10-15 minutes each grade) before the final two 10-minute rinses with absolute ethanol. From this point onward, preparation for scanning electron microscopy and transmission electron microscopy samples differed.

For the scanning electron microscopy study, samples in absolute ethanol were dried in a CO_2 critical-point drier at 31°C for 5-10 minutes, and then mounted on aluminum stubs. All samples were sputter-coated with gold prior to examination under the scanning electron microscope.

For the transmission electron microscopy study, absolute ethanol bathing the samples was replaced with propylene oxide, which was changed three times at 10-minute intervals. Samples were then embedded in Araldite CY212 mixture/propylene oxide for 1 hour, and subsequently in complete Araldite CY212 mixture overnight in a vacuum desiccator. The mixtures embedding the samples were allowed to polymerize at 60°C for 2 days prior to sectioning. Sections were stained with 4% uranyl acetate for 30 minutes and lead citrate for 5 minutes. Photomicrographs of samples were taken at 75 kV using a transmission electron microscope.

We assessed the morphological appearance of nondilated and dilated groups separately; that is, nondilated arteries and dilated arteries were compared to normal arteries. Based on electron micrographs, a pairwise semiquantitative comparison of morphological differences between nondilated and normal arteries and dilated and normal arteries for each dog was performed by three independent researchers blinded to specimen identification. Specific features in scanning electron micrographs of the intact vessel wall were identified as follows: degree of luminal narrowing, wall thickness, and corrugation of tunica intima and internal elastic lamina (IEL). For transmission electron microscopy of vessel cross-sections, observations included the degree of thinning of the entire vessel wall and its component layers, stretching and breakage of the IEL, straightening, stretching and surface rippling of smooth muscle cells, and the amount of endothelial changes such as cell loss and flattening of the luminal surface.

Statistical Analysis

For the pharmacological study, comparisons between groups at each concentration for each vasoconstrictor or vasodilator was assessed using one way analysis of variance (ANOVA), followed by a Scheffe's test of multiple comparisons if a significant probability was reached. The same method was used to compare group data of vessel lumen diameters measured with a micrometer. For the angiographic study, comparisons within a group over time were made using a paired t-test. Data were expressed as mean $\pm$ standard error (SE) of the mean. A probability level of less than 0.05 was considered significant.

Equipment and Supplies

Force-displacement transducers (model FT.03) and a polygraph (model 7D) were obtained from Grass Instrument Co., Quincy, Massachusetts. The critical-point dryer was manufactured by Seevac, Inc., Hialeah, Florida. The sputter coater, model S150B, was manufactured by Edwards Vacuum, West Sussex, England. The scanning electron microscope (model S-2500) and the transmission electron microscope (H-7000) were obtained from Hitachi Ltd., Tokyo, Japan.

RESULTS

Angiographic Measurement of Vessel Diameter

In the absence of arterial dilation with TBA, the placement of a clot of autologous blood around the artery resulted in a highly significant constriction of the ICA. On Day 7 after clot placement, the angiographic vessel diameters in the nondilated group were 48% to 89% of their original size, with a mean reduction to 63±3% ($P<0.05$) (Figure III-2B). Dilation by TBA had an immediate effect on arterial diameter. On Day 0, immediately after TBA and before clot placement, the angiographic vessel diameters in the dilated group were 148% to 200% of their original size, with a mean of 166±6% ($P<0.05$) (Figure III-3B). On Day 7 after angioplasty and clot placement, the angiographic vessel diameters in the dilated group showed that they remained dilated, with a diameter of 125% to 203% of their original size, giving a mean of 164±9% ($P<0.05$) (Figure III-3C). Similar results were obtained when the actual vessels were measured with a micrometer. On Day 7 after clot placement the measured vessel diameters in the nondilated group, were 51% to 97% the size of normal vessels, with a mean of 69±2% ($P<0.05$), while vessels which had been dilated with TBA on day 0 had a diameter of 120% to 163% of control, with a mean of 144±2% ($P<0.05$).

Thus, these results show that periarterial clot placement around segments of canine cervical ICAs results in significant reduction of vessel diameter after 7 days and that angioplasty of arterial segments immediately before the clot placement results in a vasodilatation that is sustained for 7 days even in the presence of surrounding clot.

Pharmacological Effects of Vasoconstrictor Agents

Responses to a single dose of KCl (60 mmol/L), and cumulative dose-response curves for noradrenaline, 5-HT, and $PGF_{2\alpha}$ were recorded for the three groups of vessels: normal, nondilated, and dilated.

KCl

Dilated vessels showed significantly diminished responses in comparison to nondilated and normal vessels when exposed to KCl at 60 mmol/L. The responses of

nondilated vessels in comparison to normal vessels was diminished, but the results did not reach statistical significance. These results are shown in Figure III-4A.

Noradrenaline

Dilated vessels showed significantly diminished responses in comparison to nondilated and normal vessels when exposed to NA at concentrations of 10^{-8} to 10^{-5} mol/L. The responses of nondilated vessels in comparison to normal vessels were slightly diminished at all concentrations, but the diminution did not reach statistical significance at any point. These results are shown in Figure III-4B.

5-HT

Dilated vessels showed significantly diminished responses in comparison to nondilated and normal vessels when exposed to 5-HT at concentrations of 10^{-7} to 10^{-5} mol/L, while at the lower concentrations of 10^{-8} and 10^{-9} mol/L the diminution was present but only significant when the comparison was between dilated and nondilated vessels. As in the case of noradrenaline, the responses of nondilated vessels in comparison to normal vessels were slightly diminished at concentrations of 10^{-7} to 10^{-5} mol/L, but not at a statistically significant level. These results are shown in Figure III-4C.

$PGF_{2\alpha}$

The responses of vessels in all study groups was very small. There were no responses of any arterial rings at the lower concentrations of 10^{-9} and 10^{-8} mol/L, nor was there any significant difference between normal vessels and those exposed to blood with prior TBA at any concentration. Vessels in vasospasm appeared to be more responsive than control or dilated vessels, and this reached statistical significance at a concentration of 10^{-6} mol/L. These results are shown in Figure III-4D.

Summary

Although the responses to $PGF_{2\alpha}$ differ from those to other vasoconstrictor agents in that they are smaller, and vasospasm seems to result in enhanced responsiveness to $PGF_{2\alpha}$ in this model, the observation that TBA pretreatment reduces the reactivity of arteries surrounded by perivascular clot is common to all vasoconstrictors tested. The observation that the reduction in response of nondilated (vasospastic) arteries compared with normal arteries (when tested with KCl, noradrenaline, and 5-HT) did not reach statistical significance is consistent with other models of vasospasm (30).

Pharmacological Effects of Vasodilators

After tonic contraction with noradrenaline (10^{-5} mol/L) had been established, cumulative dose-response curves for the calcium ionophore A_{23187} and bradykinin as well as the response to a single dose of papaverine (5×10^{-4} mol/L) were recorded for the three groups of vessels. In most cases, pretreatment with noradrenaline produced a large enough tonic contraction in dilated vessels to allow comparative percent relaxation data to be obtained.

Calcium Ionophore A_{23187}

Dilated vessels showed significantly diminished relaxations in comparison to nondilated and normal vessel when exposed to the calcium ionophore A_{23187} at concentrations of 10^{-6} and 10^{-5} mol/L. The responses of normal vessels in comparison to nondilated vessels were slightly diminished at all concentrations, but not at a statistically significant level. This is consistent with other models of vasospasm (30). These results are shown in Figure III-5A.

Bradykinin

There was no relaxation to bradykinin seen in vessels in the dilated group at any concentration, while the responses of nondilated and normal vessels was minimal. There was no statistically significant difference amongst the responses of the vessels in any of the groups at any concentration. These results are shown in Figure III-5B.

Papaverine

All vessel groups showed 100% relaxation after exposure to papaverine (Figure III-6).

Summary

These studies suggest that, at least in the case of calcium ionophore A_{23187} (10^{-7} to 10^{-5} mol/L), an endothelium-dependent vasorelaxant, vessels treated with periarterial blood but first dilated with TBA have an impaired response compared with vessels treated similarly but not dilated on day 0. However, endothelium-independent relaxation to papaverine is preserved even in dilated vessels.

Changes Observed With Scanning Electron Microscopy

Photomicrographs of normal, dilated, and nondilated vessels are shown in Figure III-7. Scanning electron microscopy of nondilated vasospastic vessels showed moderate diminution of the vessel lumen, decreased luminal diameter/wall thickness diameter ratio (Figure III-7B, bottom), corrugation of the internal elastic lamina (IEL), and folding of the endothelial surface (Figure III-7B, top). Similar observations of dilated vessels showed moderate enlargement of the vessel lumen (Figure III-7C, bottom), patchy endothelial denudation and straightening and thinning of the internal elastic lamina (Figure III-7C, top). Scanning electron micrographs of normal vessels are shown in Fig III-7A (top and bottom).

Changes Observed With Transmission Electron Microscopy

Photomicrographs of normal, nondilated, and dilated vessels are shown in Figure III-8. Results were consistent with those obtained by scanning electron microscopy; nondilated vasospastic vessels showed corrugation of the IEL, folding of the endothelial surface, and thickening of the vessel wall, especially the tunica media (Figure III-8B). There was some swelling and vacuolation of endothelial cells with cellular rounding and some cell separation. Rounding of smooth muscle cells with surface rippling and occasional breaks in the IEL were also noted. Transmission electron microscopy of

dilated vessels showed flattening of the endothelial cells; straightening, thinning, and occasional rupturing of the IEL; and straightening and crowding of smooth muscle cells in the tunica media (Figure III-8C). A transmission electron micrograph of a normal vessel is shown in Figure III-8A.

DISCUSSION

Use of TBA for Cerebral Vasospasm

Conventional treatment for symptomatic cerebral vasospasm has usually involved the use of hypervolemic and hypertensive therapy. However, recent advances in interventional neuroradiology have allowed for the development of newer therapeutic modalities. One technique that has gained favor since its first description by Zubkov (29) in 1984 is transluminal balloon angioplasty (TBA). It is successful in reversing the clinical effects of vasospasm in approximately 75% of selected patients (2,9,23) and generally produces results that are immediate and long-lasting. Aneurysmal repair is a prerequisite for the use of TBA. The technique is not without complications, including vessel rupture and occlusion (18). At present TBA is generally reserved for cases of moderate to severe symptomatic vasospasm that have been refractory to conventional treatment measures. However, it is not clear that these are the ideal conditions under which TBA should be employed, and earlier implementation may optimize patient outcomes. In a retrospective study, Coyne et al. concluded that the best results of angioplasty occurred when it was performed within a short time of the onset of symptoms (6). Little benefit was seen in patients of poor clinical grade or when a new neurologic deficit had become established. They suggested that angioplasty be considered if hypervolemic, hypertensive therapy did not reverse the symptoms of vasospasm within 6 to 12 hours.

Canine High Cervical Carotid Artery Model of Vasospasm

In order to perform prospective, time-course studies of vasospasm, in vivo animal models have been developed. These have involved the use of cerebral arteries in animals including the cat (35), dog (30,36) and monkey (37-40). The advantages of these models

include the presence of a cisternal space into which autologous blood can be introduced and retained, and the fact that they use intracranial cerebral arteries, thus facilitating comparison with SAH in humans. However, because of their small size and intracranial location, it is sometimes difficult to access these arteries in the course of endovascular techniques such as TBA. This has led to the development of models of vasospasm using extracranial arteries such as the femoral artery of the rat (33) and the cervical carotid artery of the rabbit (31,32,34). These models can contribute valuable information but it is important to remember that there are morphological and pharmacological differences between extracranial and intracranial arteries (41,42) and that some caution must be used when extrapolating results obtained from experiments involving these arteries to human cerebral arteries. Despite our concerns about these limitations, we have developed a model of vasospasm in the canine high-cervical carotid artery. This technique produces angiographic and morphological vessel constriction after seven days in a consistent and reproducible fashion. The vessels under study are of approximately the same caliber as the larger cerebral arteries at the base of the human brain and, most important from the perspective of this study, the vessels are suitable for endovascular manipulation.

In Vivo Mechanism of Action of TBA

There is accumulating evidence, both in extracranial (31,32,43-45) and in intracranial (30,46) arteries, that transluminal balloon angioplasty results in sustained arterial dilatation through a mechanism of smooth-muscle cell injury and paralysis. This is consistent with other evidence that vasospasm represents active smooth-muscle cell contraction (47) and that it can be acutely reversed in the presence of vasodilators such as papaverine (48,49). The results of our study suggest that in vivo balloon angioplasty performed immediately prior to the induction of vasospasm prevents vasoconstriction after SAH and produces a functional impairment in vascular reactivity that is sustained for at least seven days. These results are consistent with our previous report on the effects of immediate in vitro TBA (30) and those of others investigating in vivo TBA performed after SAH (31,32,34).

High millimolar concentrations of potassium chloride produce smooth muscle cell contraction through electromechanical coupling mechanisms with depolarization of the sarcolemma (50); whereas noradrenaline, 5-HT, and $PGF_{2\alpha}$, all act through pharmacomechanical coupling mechanisms with activation of second messengers such as 1,2-diacylglycerol and inositol 1,4,5-trisphosphate (50-52). The results of the present experiment indicate that both of these mechanisms are affected. After 7 days, normal arteries subjected to TBA showed responses to KCl, noradrenaline, and 5-HT that were markedly attenuated when compared to vasospastic and normal arteries. This suggests a sustained functional impairment of smooth muscle cell function that does not reverse rapidly. The precise nature of this impairment cannot be determined from these experiments but may involve altered calcium homeostasis that prevents the intracellular signalling leading to contraction.

The minimal response of all vessel groups to the presence of $PGF_{2\alpha}$ is in contrast to previous results found in preparations of other canine cerebral arteries (30). It is possible that the canine cervical carotid artery expresses fewer $PGF_{2\alpha}$ receptors than the canine basilar artery. The persistence of vasospasm in arteries with a rather weak response to prostaglandin $F_{2\alpha}$ would appear to provide additional evidence against a pivotal role for this compound in cerebrovascular spasm.

The calcium ionophore A_{23187} and bradykinin produce vasorelaxation via different endothelium-dependent mechanisms. Common to both mechanisms is the elevation of intracellular calcium within endothelial cells. This leads to the stimulation of nitric oxide synthase (NOS) which converts L-arginine to nitric oxide (NO), a potent vasodilator. The NO then rapidly diffuses both within the endothelial cells and across membranes to nearby smooth-muscle cells, where it interacts with soluble guanylyl cyclase, to cause relaxation (53-55). The calcium ionophore A_{23187} directly promotes entry of calcium across the endothelial cell membrane (54), whereas bradykinin acts on membrane receptors of endothelial cells and, through a signal transduction mechanism, promotes increase of cytosolic calcium and subsequent entry of calcium across the plasma membranes of endothelial cells (53-55). In the present experiments the response of dilated vessels to the calcium ionophore was attenuated in comparison to the responses

of vasospastic and normal arteries. This may be because the patchy endothelial damage seen with scanning and transmission electron microscopy after TBA, reduces the amount of NO generated and/or because there is diminished NO synthesis and release in the remaining endothelium. Also, the responsiveness to NO of the guanylyl cyclase in the smooth muscle cells of dilated vessels may be reduced.

The minimal vasorelaxant response of all vessel groups to bradykinin is in contrast to previous results found in other canine cerebral arteries (30). In blood vessels, bradykinin actually has two components to its action, a B1-receptor mediated vasoconstriction which arises from activation of the smooth muscle cells, and a B2-mediated relaxation which is endothelium dependent (56,57). In most cerebral vessels, the second response predominates, but it is possible that in the ICA, the effects are in close balance resulting in little change in tension in response to bradykinin.

Endothelium-independent relaxation to papaverine is preserved after TBA. This agent has rapid and profound effects on most smooth muscle cells, and it not surprising that the residual response to vasoconstrictors was reversed by papaverine.

It has been suggested that vasospastic arteries may remain dilated after TBA because angioplasty disrupts arterial wall components such as smooth muscle cells, myofibroblasts or the extracellular matrix thereby causing a mechanical impairment of contraction (24,58). The results of this experiment indicate that TBA causes alterations in the IEL, tunica intima, and tunica media, but that it does not cause frank disruptions in those layers. These results are generally consistent with previous in vitro and in vivo studies (30-32,46,59,60). However, although no gross mechanical disruptions of the vessel wall were seen, it is possible that the smooth muscle cell functional impairment observed represents a cellular or subcellular mechanical disruption. Indeed it may be that after TBA the artery consists of different cell populations, some of which may be approximately normal in function, while others have gross impairment of normal function.

A number of questions remain to be answered. The work reported here used a considerable dilatation of the artery. This was done because it was initially thought that the higher balloon pressures were required to dilate the thicker walled carotid artery and

because it was possible to actually observe the process of dilatation through the operating microscope. The results were entirely successful in preventing the development of cerebrovascular spasm, but it is certainly possible that a less forceful dilatation would have been equally effective and perhaps safer. Studies of varying the effects of TBA using various levels of distension or duration have important clinical implications. Early results of work from our laboratory, in which lower balloon dilatation pressures (101.3 to 202.6 kPa) were used, are similar to those reported here. The protocol described here did not contain a study group in which TBA was performed on arteries without subsequent blood clot placement. Current experiments in our laboratory include such a group, and preliminary results indicate a pharmacological impairment similar to that observed in dilated arteries surrounded with blood clot. Finally, we do not yet know long-term effects in normal arteries that have been distended by TBA, and the 7-day period studied here should be extended to examine the effects weeks or months later, to determine whether normal function returns.

In conclusion, this canine in vivo model of vasospasm using the ICA produces consistent and reproducible vasospasm, as demonstrated both by angiography and by direct measurement of vessel caliber. This region of the cerebral circulation is readily accessed by catheter and is thus suitable for studies of TBA. In this model, TBA performed prior to the induction of vasospasm prevents its development, lending experimental support to the growing clinical evidence that balloon angioplasty may have optimal benefit when performed early in the course of symptomatic vasospasm. Examination of the pharmacological and gross morphological changes present 7 days after TBA indicates a greater impairment in vasoreactivity than structural disruption of the vessel wall. Balloon dilatation and stretching of the arterial wall, which can be observed morphologically on scanning electron microscopy, result in an alteration of smooth-muscle contraction and vasodilation that is sustained for at least 7 days. Further studies are required to determine if and when vessels subjected to angioplasty return to normal contractile behavior.

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A version of this chapter has been published. Megyesi JF, Findlay JM, Vollrath B, Cook DA, Chen MH. Stroke. 1997;28:1216-1224.

STUDY DESIGN

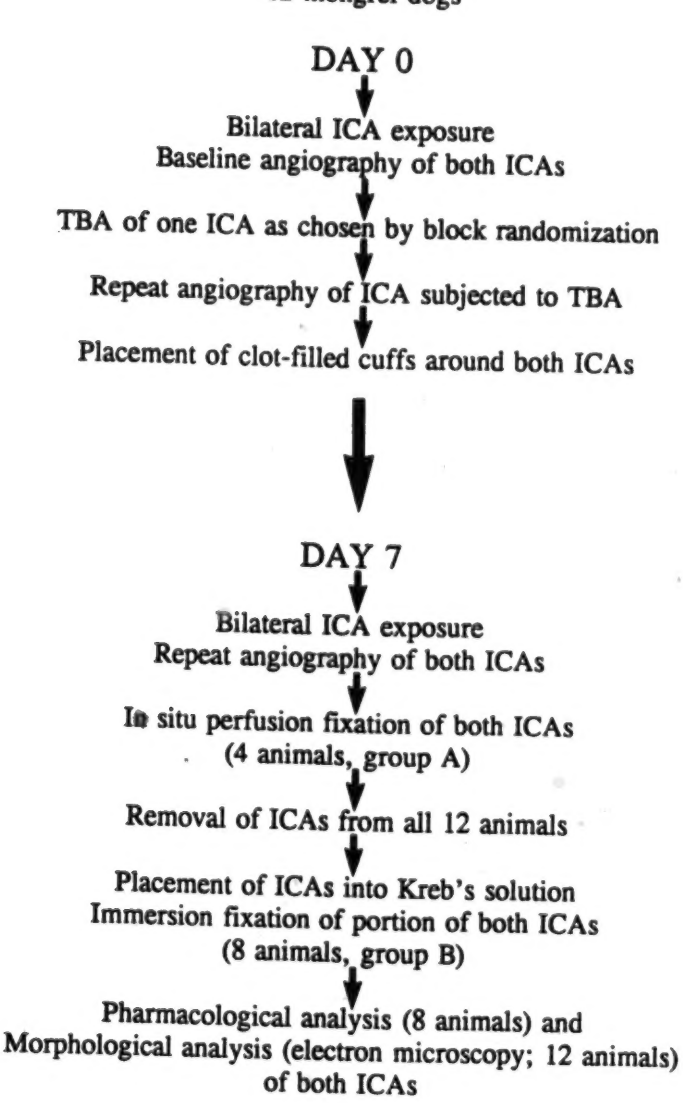
12 mongrel dogs
DAY 0
Bilateral ICA exposure
Baseline angiography of both ICAs
TBA of one ICA as chosen by block randomization
Repeat angiography of ICA subjected to TBA
Placement of clot-filled cuffs around both ICAs
DAY 7
Bilateral ICA exposure
Repeat angiography of both ICAs
In situ perfusion fixation of both ICAs
(4 animals, group A)
Removal of ICAs from all 12 animals
Placement of ICAs into Kreb's solution
Immersion fixation of portion of both ICAs
(8 animals, group B)
Pharmacological analysis (8 animals) and
Morphological analysis (electron microscopy; 12 animals)
of both ICAs

Figure III-1
Study design for the experiment to determine if transluminal balloon angioplasty performed prior to blood-clot placement can prevent the development of vasospasm in the canine high cervical carotid artery model of vasospasm. ICA=internal carotid artery, TBA=transluminal balloon angioplasty.

Figure III-2
Representative angiograms of nondilated vasospastic vessel on day 0 (A) and day 7 (B). Note extensive vasospasm (arrows) after arterial segment encased in blood clot-filled silicone elastomer cuff for 7 days.

Figure III-3
Representative angiograms of dilated vessel on day 0, before angioplasty (A); day 0, after angioplasty (B); and day 7, after angioplasty (C). Note that diameter of dilated arterial segment remains enlarged (arrows) despite the presence of blood clot-filled silicone elastomer cuff for 7 days.

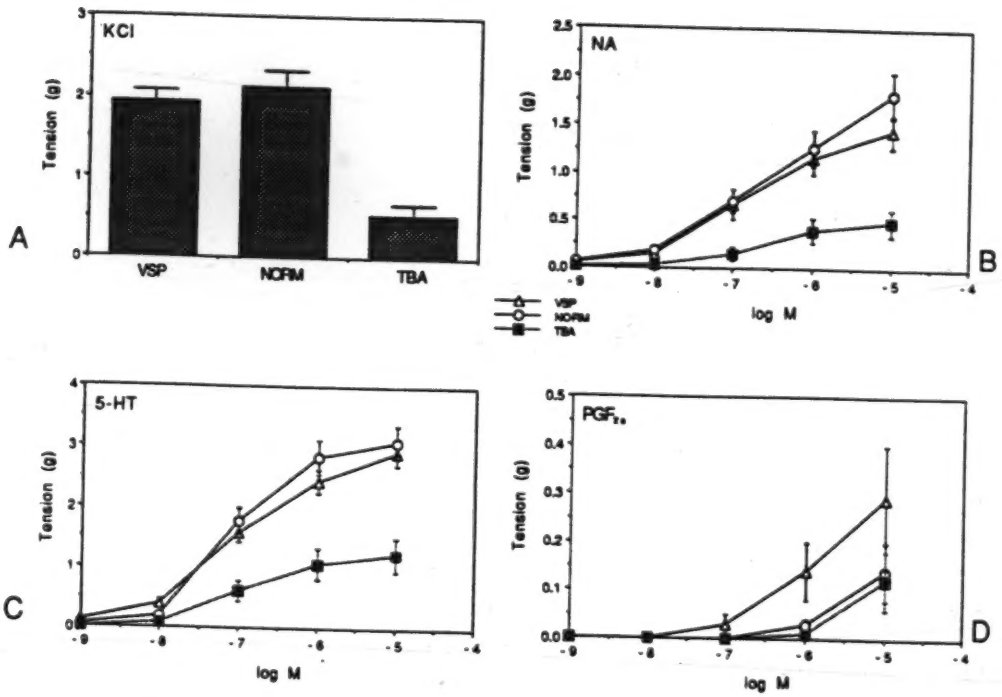

Figure III-4
Concentration-response curves of the three vessel groups (normal control [NORM], nondilated [VSP], and dilated [TBA]) to the four vasoconstrictors: potassium chloride (KCl) (A); noradrenaline (NA) (B); serotonin (5-HT) (C); prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (D). One concentration of KCl (60 mmol/L) was tested. For KCl at 60 mmol/L and for noradrenaline and 5-HT at higher concentrations, the dilated vessels showed a significant reduction in vasoconstriction compared with nondilated and normal control vessels ($P < 0.05$). The trend was present for $PGF_{2\alpha}$ but did not reach statistical significance. Exact comparisons are described in the text. M indicates vasoconstrictor concentration (molar).

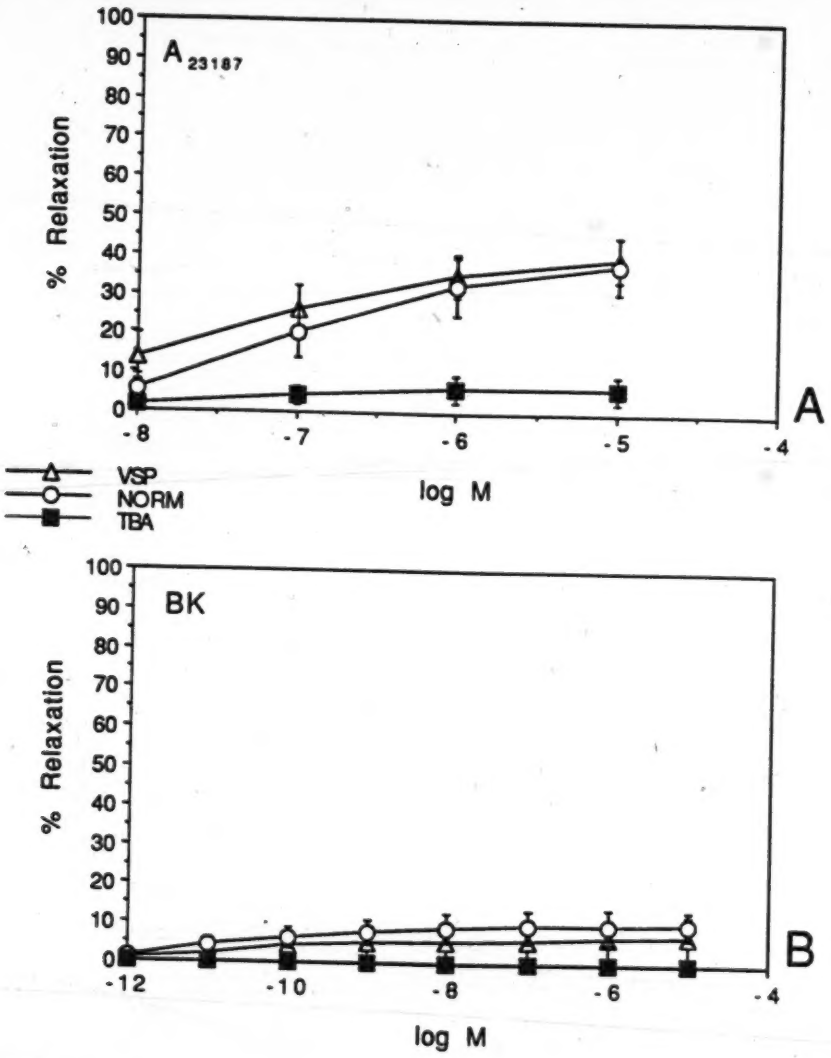

Figure III-5

Concentration-response curves of the three vessel groups (normal control [NORM], nondilated [VSP], and dilated [TBA]) to the two endothelium-dependent vasorelaxants: calcium ionophore A_{23187} (A_{23187}) (A), and bradykinin (BK) (B) . Percent relaxation was calculated using tonic contraction with 10^{-5} mol/L noradrenaline as 0% relaxation (actual values for tonic contractions to noradrenaline were: normal control arteries, 1.58 $\pm$ 0.25 g; nondilated arteries, 1.27 $\pm$ 0.12 g; dilated arteries, 0.55 $\pm$ 0.08 g). For the calcium ionophore at higher concentrations the dilated vessels showed significant reduction of endothelium-dependent vasorelaxation compared to nondilated and normal control vessels ($P < 0.05$). Dilated vessels exhibited no response to bradykinin, while non dilated and normal control vessels responded only minimally; when groups were compared there was no significant difference. Exact comparisons are described in the text. M indicates vasorelaxant concentration (molar).

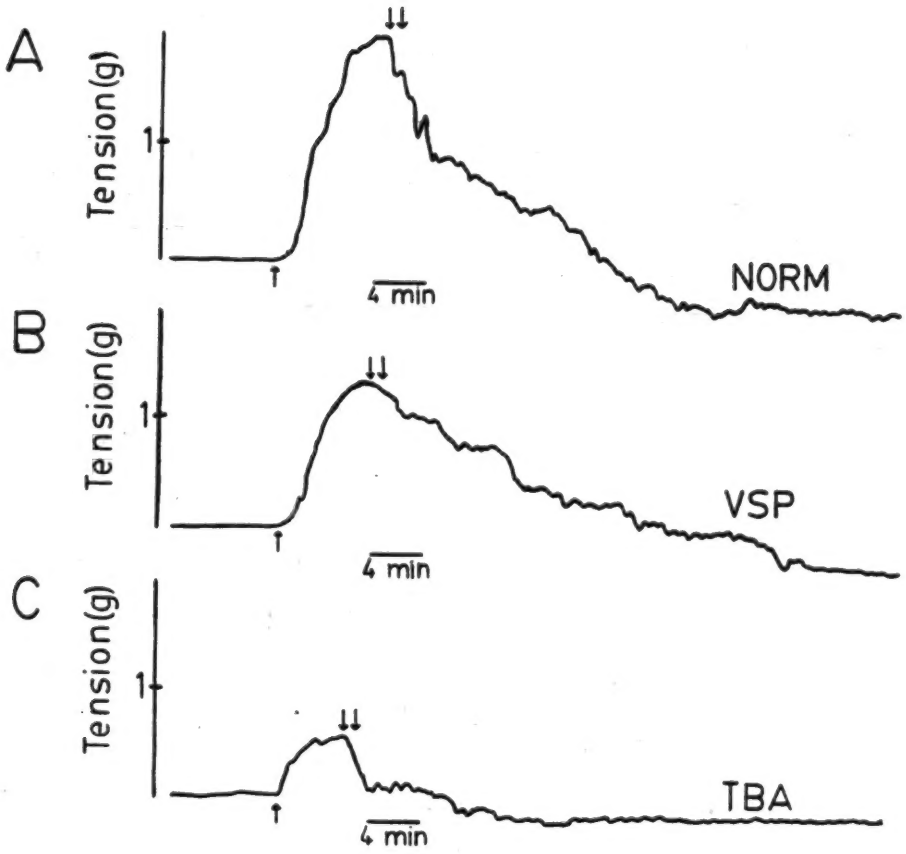

Figure III-6
Responses of representative ring preparations from each of the three vessel groups - normal control (NORM) (A), nondilated (vasospastic; VSP) (B), and dilated (TBA) (C) - to the addition of 5 x 10^{-4} mol/L papaverine (administered at the double arrow) after tonic contraction with the use of 1 x 10^{-5} mol/L noradrenaline (administered at the single arrow) had been established. In all cases curves returned to baseline levels or below, indicating 100% relaxation of the vessels.

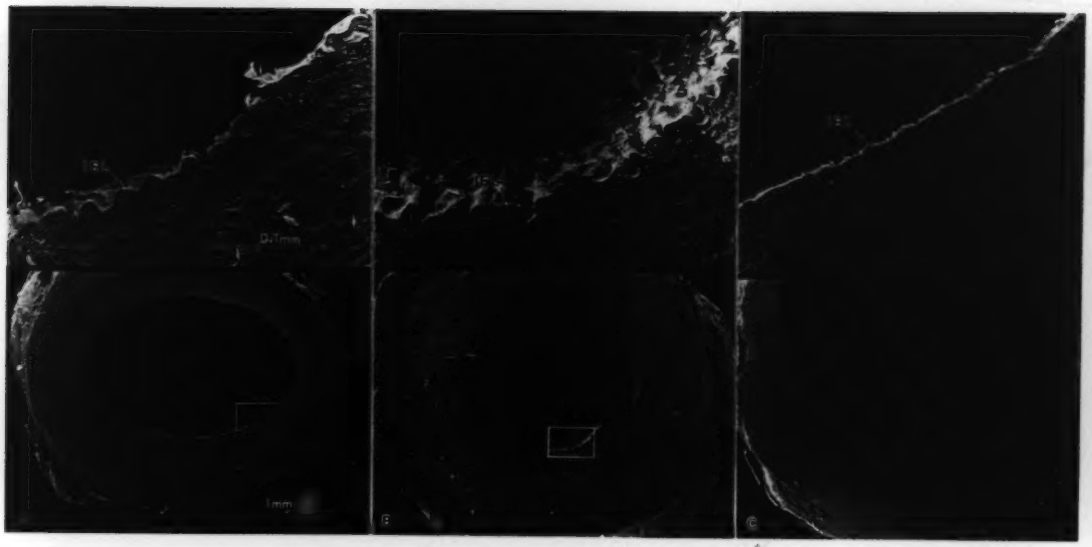

Figure III-7
Scanning electron micrographs show differences between the three vessel groups. A, Normal control vessel segment (upper panel is high power of lower). B, Nondilated vasospastic vessel segment shows smaller lumen, decreased luminal/wall diameter thickness ratio, corrugation of the internal elastic lamina (IEL), and folding of the endothelial surface (upper panel is high power of lower). C, Dilated vessel segment shows enlarged lumen, straightening and thinning of the internal elastic lamina (IEL), and patchy endothelial denudation (upper panel is high power of lower).

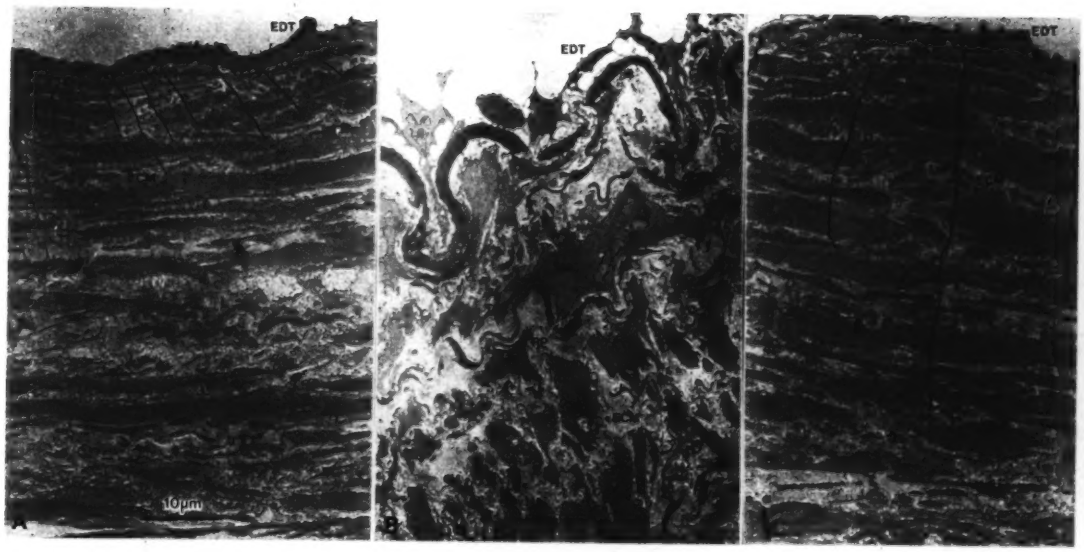

Figure III-8
Transmission electron micrographs show differences between the three vessel groups as seen in cross section of the vessel wall. A, Normal control vessel shows endothelial cells (EDT), internal elastic lamina (IEL), smooth muscle cells (SMC), and extracellular matrix (ECM). B, Nondilated vasospastic vessel shows rounding and folding of the endothelial cells (EDT), corrugation of the internal elastic lamina (IEL), and rounding with surface rippling (RP) of smooth muscle cells (SMC) within the tunica media. C, Dilated vessel shows flattening of endothelial cells (EDT); straightening, thinning, and occasional rupturing (arrowhead) of the internal elastic lamina (IEL); and straightening (smooth border [SB]) and crowding of smooth muscle cells in the tunica media.

CHAPTER FOUR
LONG-TERM PHARMACOLOGICAL AND MORPHOLOGICAL EFFECTS OF *IN VIVO* BALLOON ANGIOPLASTY IN A CANINE CAROTID ARTERY MODEL OF VASOSPASM

INTRODUCTION

Transluminal balloon angioplasty (TBA) is used to treat cerebral vasospasm following aneurysmal subarachnoid hemorrhage (1-31). Although TBA is generally reserved for cases of vasospasm refractory to conventional medical management, there is increasing evidence that under certain circumstances its use earlier in the course of vasospasm may optimize patient benefits (5,30,31). TBA results in immediate dilatation of the arterial lumen, detectable by conventional angiography (30,31). *In vitro* and *in vivo* animal studies indicate that TBA also causes an immediate and profound functional impairment of the vascular smooth muscle located within the vessel wall, as well as causing immediate morphological alterations of the vessel wall, characterized by vessel wall stretching (32-38). The long term effects of TBA have not yet been fully explored. There is evidence in one model of vasospasm that TBA leads to a decrease in smooth muscle contractility lasting for up to 28 days and that there may be concomitant damage to the endothelial and smooth-muscle cells located in the vessel wall (33,39). However, it has not been determined if the immediate functional and morphological effects of TBA are permanent. It would be of interest to know if the functional impairment of smooth muscle and the morphological alterations of the vessel wall observed immediately after the performance of TBA resolve and, if so, how long this takes. In order to study the long-term effects of TBA we used the canine high cervical carotid artery model of vasospasm previously established in our laboratory (32). We tested the hypothesis that the functional and morphological effects of TBA were no longer evident 7 weeks after angioplasty.

MATERIALS AND METHODS

Forty mongrel dogs of either sex weighing between 13 and 28 kg were used in this study. The protocol was evaluated and approved by the University of Alberta Animal and Ethics Review Committee and experiments were conducted with strict adherence to the standards of the Canadian Council on Animal Care.

Model of Blood Clot-Induced Vasospasm in the Canine High Cervical Carotid Artery

This is a new model used to study vasospasm that has been established in our laboratory (32). Briefly, placement of a blood clot held in place by a silicone elastomer cuff around the dog high cervical internal carotid artery (ICA), results in typical angiographic and structural vasospasm 7 days later, while placement of a silicone elastomer cuff alone does not significantly affect the vessel wall. For this study the following surgical protocol was used (Figure IV-1):

Animals were anesthetized with sodium pentobarbital (0.5 ml/kg) and intubated on Day 0 for angiography and blood-clot placement. Adequate anesthesia was maintained by administration of intravenous boluses of pentobarbital (0.05 ml/kg). The animals were allowed to breathe room air spontaneously. Previous experiments, using arterial blood gas determinations, had determined that this protocol maintained normocarbia in the animals under study. A midline cervical incision was made and both cervical ICAs were exposed and punctured with a 24 gauge angiocatheter for angiography (performed with 5 mL of iothalamate meglumine, injected at a rate of 0.75 mL/s). Using the operating microscope for magnification, 60 mm sections of both ICAs were then dissected free of adjacent tissues.

Twenty animals were allotted to Group A on Day 0. One of the ICAs, chosen by block-randomization, was placed within an empty 50 mm segment of silicone elastomer tubing cuff with a 10 mm inner diameter. The silicone elastomer tubing was secured with three silk ties along its length. Small cotton pledgets were positioned at either end of the cuff. The opposite ICA was not touched. The incision was sutured. The animals were cared for in the usual fashion for 7 days with daily neurological monitoring. There was no mortality or severe morbidity related to the procedures and

no animal developed a neurological deficit. On Day 7 the animals were again anesthetized, the cervical incision was reopened and angiography was repeated on both ICAs. The empty cuff was removed from the one ICA. TBA was performed on the uncuffed ICA by introducing a silicone rubber balloon into the ICA lumen via a small arteriotomy proximal to the area of study. The balloon, which was 5 mm in diameter and 12 mm in length when inflated (101.3-202.6 kPa pressure), was used to dilate the ICA twice for 10 seconds along a length of 50 mm. Angiography was then repeated on this artery.

Twenty animals were allotted to Group B on Day 0. In these animals each ICA was covered along its dissected segment with 10 mL of autologous arterial clotted blood, contained within a silicone elastomer tubing cuff having the same dimension and secured in the same fashion as described for Group A animals above. The incision was sutured. The animals were cared for in the usual fashion for 7 days with daily neurological monitoring. There was no mortality or severe morbidity related to the procedures and no animal developed a neurological deficit. On Day 7 the animals were again anesthetized, the cervical incision was reopened and angiography was repeated on both ICAs. The cuffs were removed from both ICAs. One of the ICAs, chosen by block-randomization, underwent TBA with the same silicone balloon and using the same technique as described for Group A animals above. Angiography was then repeated on this artery.

The 20 animals in Group A and the 20 animals in Group B were treated similarly from this point onwards. All were cared for in the usual fashion with daily neurological monitoring. There was no mortality or severe morbidity and no animal developed a neurological deficit. Four animals from Group A and four animals from Group B were killed with sodium pentobarbital (30 mg/kg) on each of the following days: Day 7 (baseline), Day 14, Day 21, Day 28 and Day 56. Angiography was performed on both ICAs in each animal prior to the animal being killed. After the animal was killed, both ICAs were immediately removed and placed into Krebs solution of the following composition: 120 mmol/L NaCl, 5 mmol/L KCl, 1.5 mmol/L $CaCl_2$, 1 mmol/L KH_2PO_4, 1 mmol/L $MgSO_4$, 25 mmol/L $NaHCO_3$, 55 mmol/L dextrose, aerated with

95% O_2 / 5% CO_2 and maintained at 37° C. In addition, a segment of ICA proximal to the original surgical field and the previously dissected ICA segment, was removed on one side from each animal to serve as a control preparation. Two rings were cut from each isolated ICA and the inner diameter of each ring was measured with a micrometer under magnification. The rings were used for pharmacological studies while the remainder of the arterial segments were prepared for morphological analysis.

The above protocol gave the following 5 study groups on each study day: (1) arteries that had been placed within empty cuffs and *not* subjected to TBA, taken from animals in Group A (cuffed non-blood coated or CUFF ONLY arteries); (2) arteries that had been placed within blood clot-filled cuffs and *not* subjected to TBA, taken from animals in Group B (cuffed blood coated undilated or VSP arteries); (3) arteries that had been placed within blood clot-filled cuffs and subjected to TBA, taken from animals in Group B (cuffed blood coated dilated or VSP+TBA arteries); (4) uncuffed arteries subjected to TBA, taken from animals in Group A (uncuffed dilated or TBA ONLY arteries); and (5) normal control arteries, taken from all animals (NORM arteries).

Angiographic Measurement of Vasospasm and the Effects of TBA.

The diameter of the carotid artery in millimeters was determined by direct measurement of the angiogram, at the point corresponding to the midpoint of the blood clot-filled or empty silicone elastomer cuff. For all study categories, the initial degree of angiographic vasospasm (if any) was determined by comparing vessel diameters on Day 0 with vessel diameters on Day 7 and calculating a percentage change in vessel diameter. For arteries undergoing TBA (VSP+TBA and TBA ONLY categories), the initial magnitude of the TBA-induced dilatation was calculated in two ways. First, the vessel diameter post-TBA on Day 7 was compared to the vessel diameter pre-TBA on Day 7 and a percentage change in vessel diameter was calculated. Second, the vessel diameter post-TBA on Day 7 was compared to the vessel diameter on Day 0 and a percentage change in vessel diameter was calculated.

Persistence of angiographic vasospasm (if any) was determined by comparing vessel diameters on Day 0 with vessel diameters on Day 14, 21, 28 and 56 and calculating a percentage change in vessel diameter.

Persistence of angiographic dilatation (if any; VSP+TBA and TBA categories) was determined in two ways. First, the vessel diameter on Day 14, 21, 28, and 56 was compared to the vessel diameter post-TBA on Day 7 and a percentage change in vessel diameter was calculated. Second, the vessel diameter on Day 14, 21, 28, and 56 was compared to the vessel diameter on Day 0 and a percentage change in vessel diameter was calculated.

Pharmacological Studies

Responses of arterial rings were recorded isometrically using force-displacement transducers connected to a polygraph. Rings of cerebral arteries were suspended between two stainless-steel hooks, under a resting tension of 1 gram in organ baths of 10 mL working volume containing Krebs bicarbonate solution maintained at 37°C and bubbled with 95% O_2 / 5% CO_2. After an equilibration period of 1 hour, during which the Krebs solution was changed every 15 minutes, the response to potassium chloride (KCl, 60 mmol/L) was recorded and preparations were washed until resting tension was again obtained. Cumulative dose-response curves for noradrenaline (10^{-9} to 10^{-5} mol/L) and serotonin (5-HT, 10^{-9} to 10^{-5} mol/L) were then recorded for each arterial ring. Tonic contraction of preparations was established using noradrenaline (10^{-5} mol/L) prior to vasorelaxation studies in which cumulative dose-response curves to the calcium ionophore A_{23187} (10^{-8} to 10^{-5} mol/L) were obtained. The response to papaverine (5×10^{-4} mol/L) was also recorded after tonic contraction using noradrenaline (10^{-5} mol/L) had been established. Preparations were washed until the resting tension had been restored, before another agent was tested. The ring preparations were studied in the organ baths for approximately 8-10 hours. At the end of each experiment the ring preparations were tested with KCl (60 mmol/L) to confirm that the responses were not different from the initial responses obtained to KCl at the beginning of the experiment.

Electron Microscopy

Segments of the intact vessel wall, luminal and cross-sectional aspects, were examined with scanning electron microscopy. All specimens were prefixed in 2.5% glutaraldehyde in 0.12 mol/L Millonig's buffer solution (pH 7.2) overnight at room temperature. After samples had been washed three times for 15 minutes each in Millonig's buffer, they were postfixed with 1% osmium tetroxide (OsO_4) in the same buffer for 2 hours. These samples were briefly washed in distilled water, and dehydrated in a graded series of ethanol solutions (50%, 60%, 70%, 80%, and 90%: 10-15 minutes each grade) before the final two 10-minute rinses with absolute ethanol. Samples in absolute ethanol were then dried in a CO_2 critical-point drier at 31°C for 5-10 minutes, and then mounted on aluminum stubs. All samples were sputter-coated with gold prior to examination under the scanning electron microscope.

Morphological appearance of vessels in the five categories were assessed separately and then compared with each other. Specific features in scanning electron micrographs of the intact vessel wall were identified as follows: degree of luminal narrowing, wall thickness, and corrugation of the tunica intima and internal elastic lamina (IEL).

Statistical Analysis

For the pharmacological study, comparisons between categories at each concentration for each vasoconstrictor or vasodilator was assessed using one way analysis of variance (ANOVA), followed by a Scheffe's test of multiple comparisons if a significant probability was reached. The same method was used to compare category data of vessel lumen diameters measured with a micrometer. For the angiographic study, comparisons within a category over time were made using a paired t-test. Data were expressed as mean $\pm$ standard error (SE) of the mean. A probability level of less than 0.05 was considered significant.

Equipment and Supplies

Force-displacement transducers (model FT.03) and a polygraph (model 7D) were obtained from Grass Instrument Co., Quincy, Massachusetts. The critical-point dryer was manufactured by Seevac, Inc., Hialeah, Florida. The sputter coater, model S150B, was manufactured by Edwards Vacuum, West Sussex, England. The scanning electron microscope (model S-2500) was obtained from Hitachi Ltd., Tokyo, Japan.

RESULTS

Angiographic Measurement of Vessel Diameter (Figure IV-2)

CUFF ONLY arteries

Arteries placed within empty cuffs did not exhibit any significant change in diameter on any day when compared to the original artery diameter on day 0 ($P > 0.05$). Mean percentage of original size was: day 7, $109 \pm 7\%$; day 14, $102 \pm 2\%$; day 21, $99 \pm 3\%$; day 28, $104 \pm 4\%$; day 56, $105 \pm 4\%$

VSP arteries

Arteries placed within blood clot-filled cuffs and not subsequently dilated exhibited a significant reduction in diameter on days 7 and 14 ($P < 0.05$), but not on days 21, 28, and 56 ($P > 0.05$). Mean percentage of original size was: day 7, $55 \pm 11\%$; day 14, $68 \pm 3\%$; day 21, $101 \pm 7\%$; day 28, $101 \pm 2\%$; day 56, $103 \pm 2\%$.

VSP+TBA arteries

Arteries placed within blood clot-filled cuffs developed significant arterial narrowing on day 7 (prior to TBA), with a mean reduction to $56 \pm 12\%$ of their original diameter ($P < 0.05$). After TBA these vessels had lumen diameters that were $89 \pm 5\%$ of their original size (not significant, but a significant dilatation of $173 \pm 10\%$ of the constricted state). On days 14, 21, 28, and 56 lumen diameters remained dilated when compared to their day 0 values (prior to TBA) but the differences were not significant. Mean percentage of original size was: day 7, $89 \pm 5\%$; day 14, $110 \pm 7\%$; day 21, $105 \pm 2\%$; day 28, $102 \pm 2\%$; day 56, $101 \pm 4\%$.

TBA ONLY arteries

Uncuffed arteries did not exhibit any significant change in diameter on day 7 (prior to TBA), having a mean diameter of 100±1% of their original diameter ($P>0.05$). After TBA these arteries exhibited a significant mean lumen dilatation to 106±2% of their original size ($P<0.05$). On days 14, 21, 28, and 56 lumen diameters of the arteries remained dilated when compared to their day 0 values (prior to TBA), but the difference was only significant on day 14. Mean percentage of original size was: day 7, 106±2%; day 14, 114±2%; day 21, 108±3%; day 28, 101±1%; day 56, 101±3%.

Micrometer Measurement of Vessel Diameter (Figure IV-3)

CUFF ONLY arteries

The measured vessel diameters of arteries placed within empty cuffs were not significantly different from the diameters of normal control arteries on any day ($P>0.05$). Mean percentage of normal artery diameter was: day 7, 100±2%; day 14, 98±2%; day 21, 100±4%; day 28, 99±3%; day 56, 99±2%.

VSP arteries

The measured vessel diameters of arteries placed within blood clot-filled cuffs and not subsequently dilated were significantly reduced compared with normal control vessels on days 7 and 14 ($P<0.05$), but not on days 21, 28 and 56 ($P>0.05$). Mean percentage of normal artery diameter was: day 7, 72±1%; day 14, 78±3%; day 21, 89±4%; day 28, 99±4%; day 56, 101±2%.

VSP+TBA arteries

The measured vessel diameters of arteries placed within blood clot-filled cuffs and one week later subjected to TBA were significantly increased compared with normal control vessels on days 7 and 14 ($P<0.05$), but not on days 21, 28, and 56 ($P>0.05$). Mean percentage of normal artery diameter was: day 7, 126±3%; day 14, 133±6%; day 21, 106±3%; day 28, 102±1%; day 56, 102±2%.

TBA ONLY arteries

The measured vessel diameters of uncuffed arteries subjected to TBA (one week after initial angiography) were increased compared to normal control vessels on days 7, 14, 21, and 28, but not at a significant level ($P>0.05$); there was no significant difference on day 56 ($P>0.05$). Mean percentage of normal artery diameter was: day 7, 119±2%; day 14, 110±3%; day 21, 111±5%; day 28, 107±5%; day 56, 95±2%.

Pharmacological Effects of Vasoconstrictor Agents

On each day of the study (day 7, 14, 21, 28, and 56) responses to a single dose of KCl (60 mmol/L) and cumulative dose-response curves for noradrenaline and serotonin (5-HT) were recorded for the five categories of vessels: normal control (NORM), cuffed non-blood coated (CUFF ONLY), cuffed blood coated undilated (VSP), cuffed blood coated dilated (VSP+TBA), and uncuffed dilated (TBA ONLY). All vessel categories exhibited cumulative dose-responses to noradrenaline and 5-HT on each day of the study. However, the data used for statistical comparison on each day were taken from the highest dose tested for each agent (10^{-5} mol/L).

KCl

The responses of arteries surrounded by an empty cuff were not significantly different from that of normal control arteries on any day when tested with KCl at 60 mmol/L. The responses of cuffed blood coated undilated (vasospastic) arteries were diminished in comparison to normal arteries on all days, but the results did not reach statistical significance. The responses of cuffed blood coated dilated arteries in comparison to normal arteries were significantly diminished on days 7, 14, and 21; on days 28 and 56 the differences were no longer significant. The responses of uncuffed dilated arteries in comparison to normal arteries was significantly diminished on day 7; on days 14, 21, 28 and 56 the differences were no longer significant. These results are shown in Figure IV-4A.

Noradrenaline

The responses of arteries surrounded by an empty cuff were not significantly different from that of normal control arteries on any day when tested with noradrenaline at 10^{-5} mol/L. The responses of cuffed blood coated undilated (vasospastic) arteries were diminished in comparison to normal arteries on all days, with the results reaching significance on days 7 and 21. The responses of cuffed blood coated dilated arteries in comparison to normal arteries were significantly diminished on days 7, 14, and 21; on days 28 and 56 the differences were no longer significant. The responses of uncuffed dilated arteries in comparison to normal arteries was significantly diminished on day 7; on days 14, 21, 28 and 56 the differences were no longer significant. These results are shown in Figure IV-4B.

5-HT

The responses of arteries surrounded by an empty cuff were not significantly different from that of normal control arteries on any day when tested with 5-HT at 10^{-5} mol/L. The responses of cuffed blood coated undilated (vasospastic) arteries were diminished in comparison to normal arteries on all days, but the results did not reach statistical significance. The responses of cuffed blood coated dilated arteries in comparison to normal arteries were significantly diminished on days 7, 14, and 21; on days 28 and 56 the differences were no longer significant. The responses of uncuffed dilated arteries in comparison to normal arteries were significantly diminished on day 7; on days 14, 21, 28 and 56 the differences were no longer significant. These results are shown in Figure IV-4C.

Summary

Normal arteries and arteries surrounded by an empty silicone elastomer cuff exhibited responses to the vasoconstrictors that were not significantly different on any day. Cuffed blood coated undilated (vasospastic) arteries exhibited diminished responses to all of the vasoconstrictors tested, but not always at a significant level. This is consistent with other models of vasospasm (35). Cuffed blood coated dilated arteries

exhibited significantly diminished responses to all vasoconstrictors on days 7, 14, and 21; on days 28 and 56 the differences were no longer significant. Uncuffed dilated arteries exhibited significantly diminished responses to all vasoconstrictors on day 7; on days 14, 21, 28, and 56 the differences were no longer significant.

Pharmacological Effects of Vasodilators

On each day of the study (day 7, 14, 21, 28, and 56), after tonic contraction with noradrenaline (10^{-5} mol/L) had been established, cumulative dose response curves for the calcium ionophore A_{23187} and the response to a single dose of papaverine (5×10^{-4} mol/L) were recorded for the five categories of vessels. In most cases pretreatment with noradrenaline produced a large enough tonic contraction in dilated vessels to allow comparative percent relaxation data to be obtained. All vessel categories exhibited cumulative dose-responses to the calcium ionophore A_{23187} on each day of the study. However, the data used for statistical comparison on each day were taken from the highest dose tested (10^{-5} mol/L).

Calcium Ionophore A_{23187}

The relaxations of arteries surrounded by an empty cuff were not significantly different from that of normal control arteries on any day when tested with the calcium ionophore A_{23187} at 10^{-5} mol/L. The relaxations of cuffed blood coated undilated (vasospastic) arteries in comparison to normal control arteries were decreased on days 7 and 14, and slightly increased on days 21, 28, and 56 but the results did not reach statistical significance on any day. The relaxations of cuffed blood coated dilated arteries in comparison to normal arteries were significantly diminished on days 7 and 14 but not on days 21, 28, and 56. The relaxations of uncuffed dilated arteries in comparison to normal arteries were diminished on every study day except day 21, but not at a significant level. These results are shown in Figure IV-5.

Papaverine

All vessel groups showed 100% relaxation after exposure to papaverine on all days.

Summary

Only cuffed blood coated dilated arteries, when compared to normal control arteries, exhibited diminished responses to the calcium ionophore A_{23187}, an endothelium-dependent vasorelaxant, that were statistically significant. This occurred on days 7 and 14; on days 21, 28, and 56 the differences were no longer significant. However, endothelium-independent relaxation to papaverine was preserved in all vessels (even those that had been subjected to dilatation) on all days.

Changes Observed With Scanning Electron Microscopy

Specific features of vessels in each of the five categories (normal [NORM], cuffed non-blood coated [CUFF ONLY], cuffed blood coated undilated [VSP], cuffed blood coated dilated [VSP+TBA], and uncuffed dilated [TBA ONLY]) were assessed using electron microscopy. A composite of photomicrographs of normal (NORM), cuffed non-blood coated (CUFF ONLY), and uncuffed dilated (TBA ONLY) vessels from each study day (7, 14, 21, 28 and 56) is shown in Figure IV-6. A composite of photomicrographs of normal (NORM), cuffed blood coated undilated (VSP), and cuffed blood coated dilated (VSP+TBA) vessels from each study day is shown in Figure IV-7.

Scanning electron microscopy of cuffed non-blood coated vessels (CUFF ONLY) did not show any features that allowed them to be clearly distinguished from normal vessels on any of the study days (Figure IV-6A,D,G,J,M): lumen diameter was not decreased, luminal/wall diameter thickness ratio was not decreased and there was no alteration of the internal elastic lamina (IEL) or endothelial surface.

Scanning electron microscopy of cuffed blood coated undilated (vasospastic) vessels (VSP) showed moderate diminution of the vessel lumen, decreased luminal/wall diameter thickness ratio, corrugation of the internal elastic laminal, and folding of the endothelial surface all of which were most prominent on days 7 and 14 (Figure IV-7B,E),

were less notable on days 21 and 28 (Figure IV-7H,K), and almost absent on day 56 (Figure IV-7N).

Scanning electron microscopy of cuffed blood coated dilated vessels (VSP+TBA) showed enlargement of the vessel lumen to near normal size, patchy endothelial denudation, and straightening and thinning of the IEL. The near normalization of the lumen size was present on each of the study days while the other changes were most prominent on days 7 and 14 (Figure IV-7C,F) , were less notable on days 21 and 28 (Figure IV-7I,L), and almost absent on day 56 (Figure IV-7O).

Scanning electron microscopy of uncuffed dilated vessels (TBA ONLY) showed slight enlargement of the vessel lumen above normal size, with some patchy endothelial denudation and straightening and thinning of the IEL that were less marked than that observed in vasospastic arteries after TBA (see above). These changes were most notable on day 7 (Figure IV-6C), less notable on day 14 (Figure IV-6F), and almost absent on days 21, 28, and 56 (Figure IV-6I,L,O).

DISCUSSION

First described by Zubkov et al. (28) in 1984 transluminal balloon angioplasty (TBA) is now used in the treatment of cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Clinical experience suggests that the effectiveness of TBA at reversing clinical and angiographic vasospasm is immediate and long-lasting (30,31). Although the complications that can occur with an interventional technique such as TBA are serious (17,30,31), in centers equipped with the proper technology and personnel it is considered an acceptably safe procedure. Clinical evidence thus far also suggests that TBA of cerebral vessels is not associated with significant or permanent damage to the vessel wall (30,31), or at least unassociated with damage that is detectable in angiograms of patients who are followed weeks or months after the procedure. This is different from coronary angioplasty where restenosis of dilated arteries has been a major problem (30,40-43). Even though the clinical evidence is already favorable, it is still useful to determine the long-term effects of TBA on the morphology and function of the arterial wall. Although a number of studies have already addressed this issue in part (33,34,39),

there is no study that examines the angiographic, morphologic and pharmacologic properties of both normal and vasospastic arteries sequentially up to 2 months after the performance of TBA.

Canine High Cervical Carotid Artery Model of Vasospasm

Previous studies using this new model have established that after seven days it reliably and reproducibly produces vasospasm that has the angiographic appearance, morphologic characteristics, and many of the functional alterations seen in cerebral vasospasm in humans (32). The results presented here corroborate this previous work. Vessels surrounded by blood filled cuffs for seven days exhibited significant angiographic arterial narrowing. The responses of the vessels to applied vasoconstrictors were diminished compared to normal vessels, a feature also found in other accepted models of vasospasm (35). Morphological analysis of the vessel wall showed characteristic features of vasospasm with narrowing of the arterial lumen, a decrease in the luminal/wall diameter thickness ratio, and corrugation and folding of the IEL and endothelial surface (35,44).

In humans the time course of vasospasm has been well established (45,46). Onset of vasospasm occurs 2 to 4 days after SAH, is maximal between days 6 and 8, may still be present at day 14, and is usually resolved by day 21 (46). The results presented here indicate that vasospasm in the canine high cervical carotid artery model follows quite closely the time course of vasospasm in man. The significant angiographic narrowing of the ICA seen on days 7 and 14 after blood clot placement had resolved by day 21. Similarly, actual vessel lumen diameters as measured by a micrometer were no longer decreased on day 21. The morphological changes characteristic of vasospasm in human arteries (and in arteries in other animal models) (44,46) were prominent in this model on days 7 and 14, were less notable by days 21 and 28, and were almost absent by day 56. The pharmacological alterations of arteries in vasospasm, in both human and animal studies, are generally less consistent than those observed with angiography or morphological examination (35,47-49). In this model responses of vasospastic arteries to vasoconstrictors were generally diminished, moreso in the first 3 weeks after blood

clot placement than after 4 to 8 weeks, but not usually at a significant level. This observation is consistent with that of other vasospasm models (35). The angiographic, morphological and pharmacological characteristics of this model, based on the results presented here and in previous work (32), indicate that it is satisfactory for long-term studies of arterial vasospasm.

Immediate Effects of TBA on Normal and Vasospastic Arteries

TBA resulted in an immediate and significant angiographic (and measurable) dilatation of both normal and vasospastic arteries. The lumens of normal arteries were increased above normal while the lumens of vasospastic arteries were significantly increased above their constricted state and were not significantly different from their baseline diameters before blood clot placement. Hence the angiographic goal of TBA was achieved in this experiment.

TBA resulted in a significant impairment in the ability of both normal and vasospastic vessels to respond to vasoconstrictor agents on the day the dilatation was performed. Macdonald et al. (33) found similar results using a rabbit carotid artery model of vasospasm. Fujii et al. (34), using a canine basilar artery model of vasospasm, found that pharmacological responses of vasospastic arteries subjected to TBA were attenuated but that the responses of normal arteries were not. TBA resulted in a significant impairment in the ability of vasospastic (but not normal) vessels to relax upon exposure to the calcium ionophore A_{23187}, an endothelium-dependent vasorelaxant. A similar observation was made by Macdonald et al. (33) using acetylcholine, another endothelium-dependent vasorelaxant.

The exact mechanism responsible for the pharmacological impairment seen immediately after TBA cannot be determined from these experiments; however there is evidence pointing to possible sites of action. KCl initiates smooth muscle cell contraction at sarcolemmal plasma membrane voltage gated calcium channels; noradrenaline and serotonin act at plasma membrane receptors to initiate an intracellular signalling cascade involving 1,2-diacylglycerol and inositol 1,4,5-trisphosphate that leads to contraction (50,51). It may be that TBA disturbs the smooth muscle cell membrane, disrupting both

voltage gated calcium channels and membrane receptors, rendering the cell less responsive to agents that act at these sites. The observation that TBA does not alter the response of smooth muscle cells to papaverine lends further support to this hypothesis. Since papaverine is lipid soluble and diffuses across the smooth muscle cell plasma membrane to act intracellularly by inhibiting phosphodiesterases, its effects should not be altered by perturbations to the cell membrane. In contrast to papaverine, vasorelaxants such as the calcium ionophore A_{23187} act by increasing nitric oxide (NO) production in endothelial cells; NO then binds to soluble guanylyl cyclase in smooth muscle cells, generating cyclic guanosine monophosphate which leads to smooth muscle cell relaxation (52). It may be that TBA alters endothelial cell function, leading to a reduction in NO synthesis, or that it alters the responsiveness of smooth muscle cells to NO (32).

Normal and vasospastic arteries had a similar appearance immediately after TBA. Alterations, such as patchy endothelial denudation and thinning of the IEL, were the same as those observed previously in this model (32), as well as in other models of vasospasm (34,35,37,39). The role of arterial wall structural alterations in the prevention of arterial reconstriction immediately after TBA is unclear. There is some evidence that disruption of smooth muscle cells, myofibroblasts, and/or the extracellular matrix may mechanically impair arterial wall contraction immediately after TBA (39,53,54). However the alterations after TBA in this model (32), and in others (34), do not appear to be severe enough, at least at a gross morphological level, to fully explain the sustained dilatation.

Long-term Effects of TBA on Normal and Vasospastic Arteries

Angiograms of normal arteries and vasospastic arteries subjected to TBA showed that they remained near normal size (or slightly larger) on all study days following performance of the procedure. The same observation has been made in humans undergoing angiography weeks or months after TBA, as well as in other animal models (30,31). Since it produces a sustained dilatation of the artery, TBA usually has an advantage over pharmacological treatments for vasospasm such as papaverine (55-57).

Vasospastic arteries showed significantly attenuated responses to vasoconstrictor agents one and two weeks after TBA (day 14 and 21) but not three and seven weeks after TBA (day 28 and 56). This indicates that the functional impairment of the vessel wall induced by TBA is effective at preventing reconstriction of the artery during the time period in which vasospasm is most likely to still occur, but that it does not result in a permanent functional alteration of the vessel wall. Both of these are desirable features of TBA. Macdonald et al. (33) also observed that angioplastied vasospastic arteries did not show attenuated responses to vasoconstrictors 28 days after TBA. It may be that after TBA the artery consists of different cell populations, some of which may be approximately normal in function, while others have a gross impairment of function (32). Over the ensuing days cellular recovery occurs with the relative amount of normal to abnormal cells increasing until after three weeks the cell population is normalized to pre-TBA levels.

Normal arteries did not show significantly decreased responses to vasoconstrictor agents except on the day TBA was performed (day 7). In this model TBA appears to have a more profound effect on vasospastic arteries than it does on normal arteries. Fujii et al. (34) observed that normal arteries subjected to TBA did not show a functional impairment as great as that seen in vasospastic arteries even one day after TBA had been performed, while Macdonald et al. (33) observed variable results depending on the day after TBA and the vasoconstrictor agent used. The reason why TBA should have a lesser effect on normal arteries than on vasospastic arteries is not clear. It may be that vasospasm causes alterations in smooth muscle cells that render them more susceptible to further perturbations that might be caused by TBA.

Vasospastic (but not normal) arteries showed significantly attenuated responses to the calcium ionophore A_{23187} one week after TBA (day 14) but not on any study day thereafter. This suggests that the functional impairment caused by TBA does not affect the vessel wall components responsible for vasorelaxation as severely as it affects the components responsible for vasoconstriction. A similar result was found by Macdonald et al. for acetylcholine (33). If impaired endothelial cell function is indeed responsible for the immediate attenuation in vasorelaxation seen after TBA, it may be that the

endothelium of angioplastied vessels recovers more quickly than the smooth muscle cells responsible for vasoconstriction.

The morphological changes seen in vasospastic arteries immediately after TBA (patchy endothelial denudation and thinning of the IEL) although still present one week after TBA (day 14) were resolving two and three weeks after TBA (day 21 and 28) and almost absent seven weeks after TBA (day 56). This observation is consistent with that of Fujii et al. (34) who noted that 50 days after TBA arteries had no residual changes. Macdonald et al. (39) noted that three to four weeks after TBA arteries exhibited endothelial proliferation and a trend for thinning of the tunica media. The morphological changes seen in normal arteries immediately after TBA, which were initially not as prominent as those seen in vasospastic arteries, were resolving one week after TBA (day 14) and almost absent two weeks after TBA (day 21). This is similar to observations made by Macdonald et al. (39) and Fujii et al. (34) and is further evidence that TBA has more of an effect on vasospastic arteries than it does on normal arteries.

In conclusion, the canine high cervical internal carotid artery model produces consistent and reproducible vasospasm that exhibits characteristic angiographic, morphologic, and pharmacologic features that follow the time course of vasospasm in humans and in other animal models of vasospasm. The accessible location of the canine ICA, the size of the vessel (similar to human intracranial internal carotid artery and M1 middle cerebral artery), and the ease with which endovascular procedures may be performed within the vessel all make it a good model for short- and long-term studies. In this model TBA performed on vasospastic arteries results in a functional impairment that lasts for two to three weeks. Morphological changes present immediately and one week after TBA in vasospastic arteries, begin to resolve two to three weeks after TBA and are almost gone seven weeks after TBA. TBA of normal vessels causes functional impairment and morphological alterations that are not as severe and not as long-lasting as those seen in vasospastic arteries. The results presented here lend experimental support to the clinical observation that TBA of vasospastic cerebral arteries does not appear to cause a permanent alteration of the vessel wall. This may provide additional reassurance to those clinicians performing the technique. Further work is required to

determine more precisely the cellular mechanisms underlying the functional alterations caused by TBA, their relationship to the observed morphological changes, and the way in which they resolve over time.

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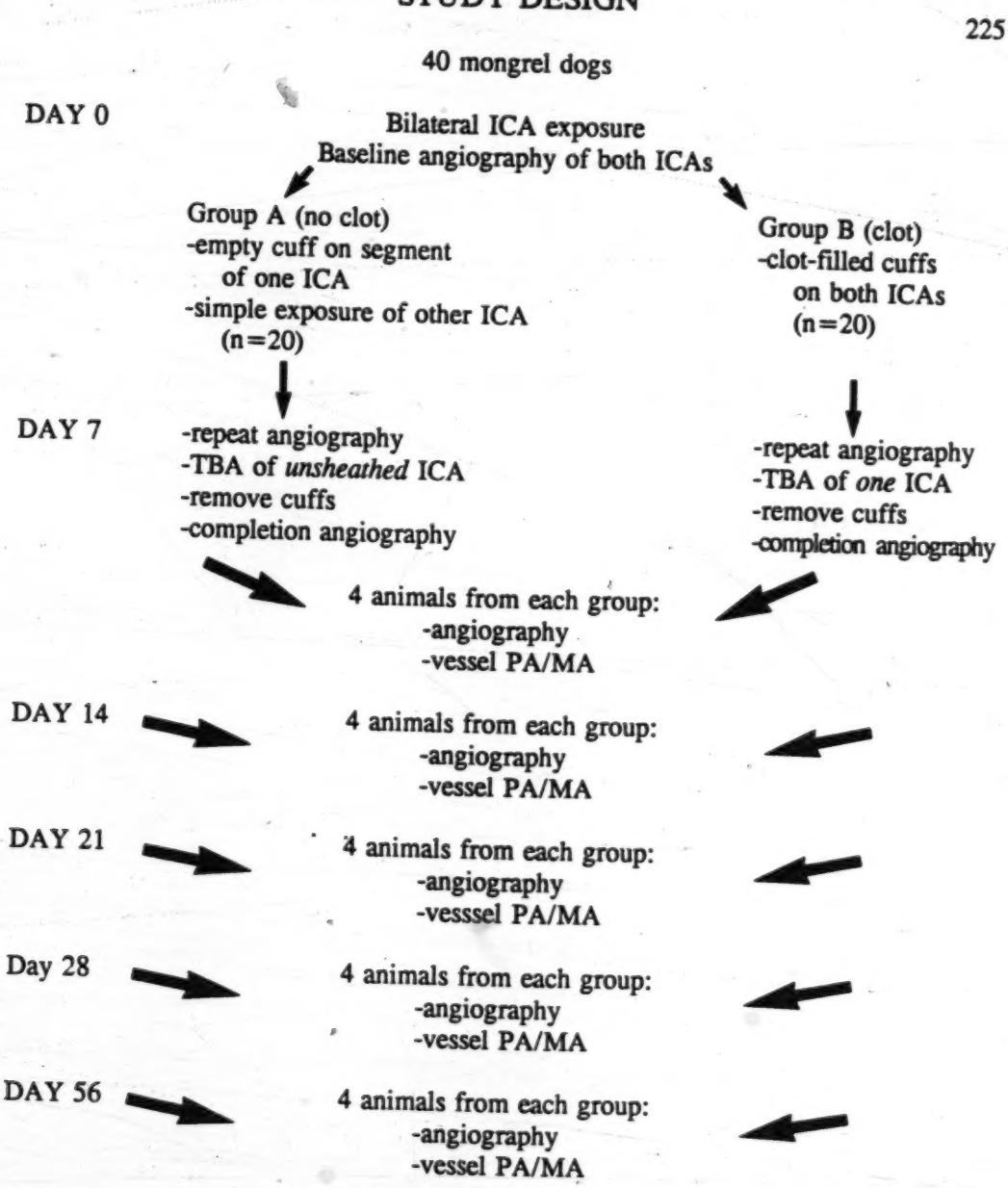

Figure IV-1
Study design for the experiment to determine the long-term angiographic, pharmacologic and morphologic effects of transluminal balloon angioplasty in the canine high cervical carotid artery model of vasospasm. ICA=internal carotid artery, TBA=transluminal balloon angioplasty, PA=pharmacological analysis, MA=morphological analysis (electron microscopy).

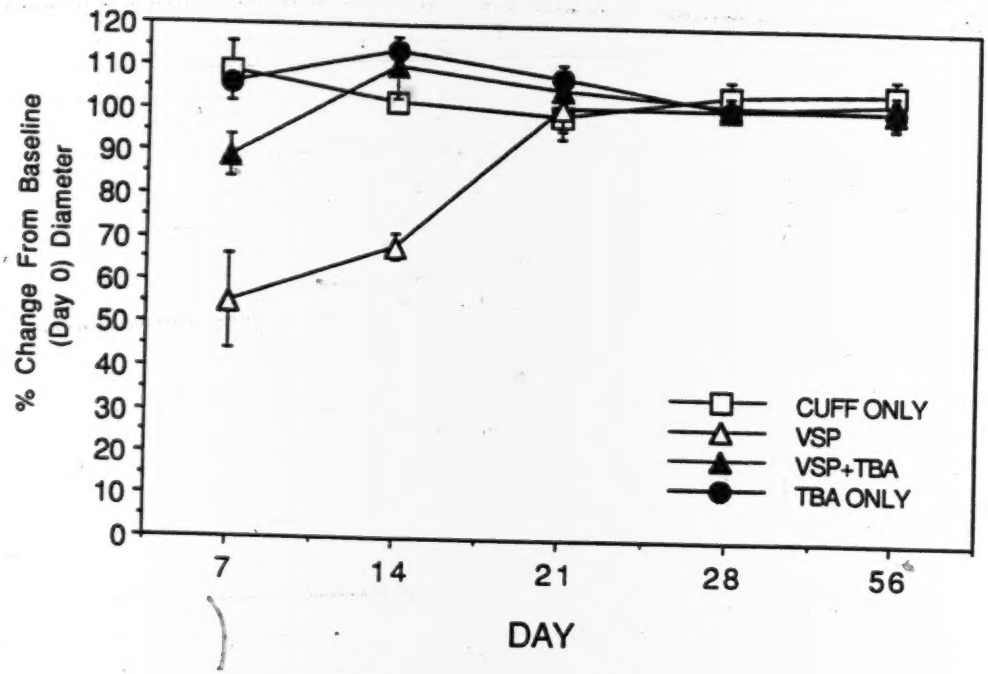

Figure IV-2

Percentage change from baseline (day 0) on each study day of angiographic vessel diameters for each of the vessel catagories (cuffed non-blood coated [CUFF ONLY], cuffed blood coated undilated [VSP], cuffed blood coated dilated [VSP+TBA], and uncuffed dilated [TBA ONLY]). Cuffed non-blood coated vessels did not show any significant change in diameter compared to baseline on any day. Cuffed blood coated undilated (vasospastic) vessels showed a significant reduction in diameter compared to baseline on days 7 and 14, but not on days 21, 28, and 56. After transluminal balloon angioplasty, cuffed blood coated dilated vessels had diameters that were $89\pm5\%$ of baseline (not significant, but a significant dilatation of $173\pm10\%$ of the constricted state); diameters were dilated compared to baseline on days 14, 21, 28, and 56, but the differences were not significant. After transluminal balloon angioplasty, uncuffed dilated arteries had diameters that were $106\pm2\%$ of baseline, a significant increase; diameters remained dilated compared to baseline on days 14, 21, 28, and 56, but the difference was significant only on day 14.

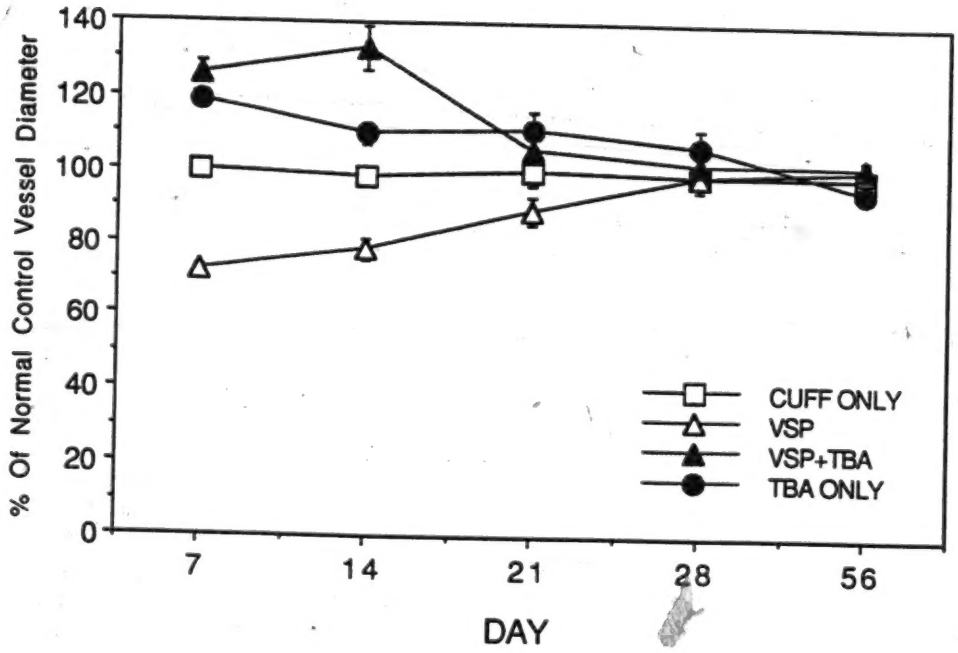

Figure IV-3
Percentage of normal control vessel inner lumen diameter as determined by measurement with a micrometer on each study day for each of the vessel catagories (cuffed non-blood coated [CUFF ONLY], cuffed blood coated undilated [VSP], cuffed blood coated dilated [VSP+TBA], and uncuffed dilated [TBA ONLY]). Diameters of cuffed non-blood coated vessels were not significantly different from diameters of normal control vessels on any day. Diameters of cuffed blood coated undilated (vasospastic) vessels were significantly reduced compared to normal control vessels on days 7 and 14, but not on days 21, 28 and 56. After transluminal balloon angioplasty, diameters of cuffed blood coated dilated vessels were significantly increased compared to normal control vessels on days 7 and 14, but not on days 21, 28, and 56. After transluminal balloon angioplasty, diameters of uncuffed dilated vessels were increased compared to normal control vessels on days 7, 14, 21, and 28, but not at a significant level; there was no significant difference on day 56.

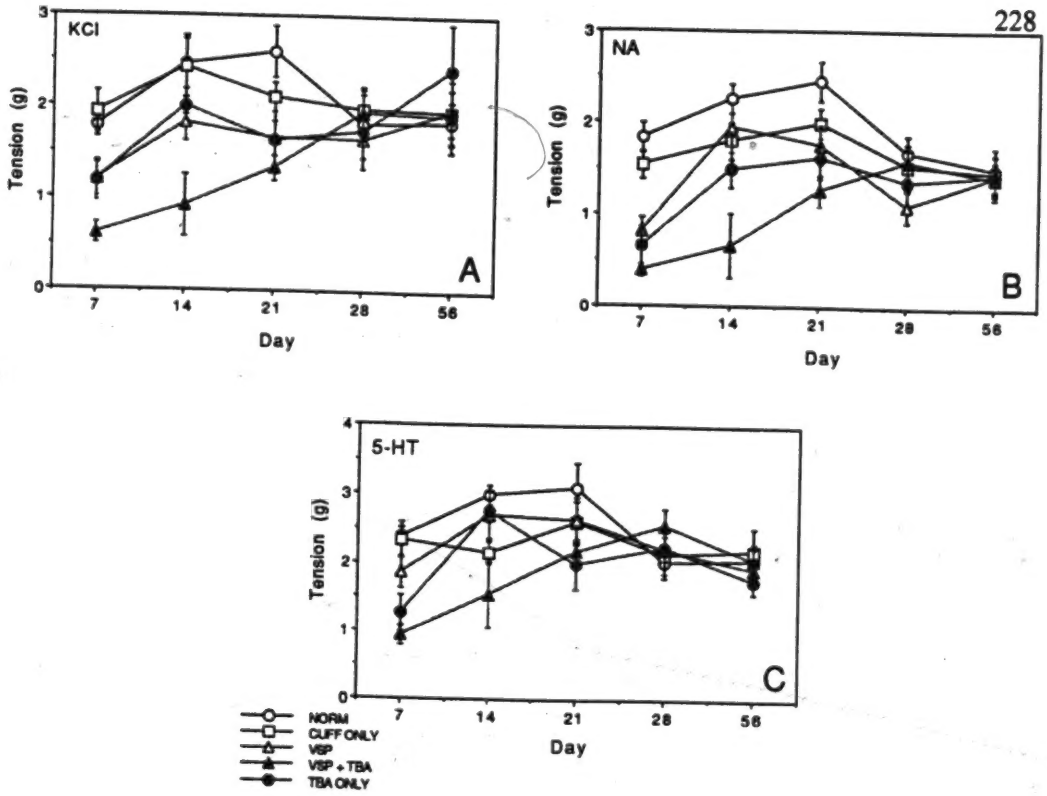

Figure IV-4

Responses on each study day of the five vessel catagories (normal control [NORM], cuffed non-blood coated [CUFF ONLY], cuffed blood coated [VSP], cuffed blood coated dilated [VSP+TBA], and uncuffed dilated [TBA ONLY]) to the three vasoconstrictors: potassium chloride (KCl) (A); noradrenaline (NA) (B); serotonin (5-HT) (C). One concentration of KCl (60 mmol/L) was tested; although concentration response-curves for noradrenaline and 5-HT were determined, only the responses to the highest concentration of noradrenaline (10^{-5} mol/L) and 5-HT (10^{-5} mol/L) on each study day are shown. The responses of normal control vessels and cuffed non-blood coated vessels were not significantly different on any day when tested with each of the vasoconstrictors. Cuffed blood coated undilated (vasospastic) vessels had diminished responses compared to normal control vessels to all vasoconstrictors on all days but not always at a significant level. Cuffed blood coated dilated vessels had significantly diminished responses compared to normal control vessels to all vasoconstrictors on days 7, 14, and 21; on days 28 and 56 the differences were no longer significant. Uncuffed dilated vessels had significantly diminished responses compared to normal control vessels to all vasoconstrictors on day 7; on days 14, 21, 28, and 56 the differences were no longer significant.

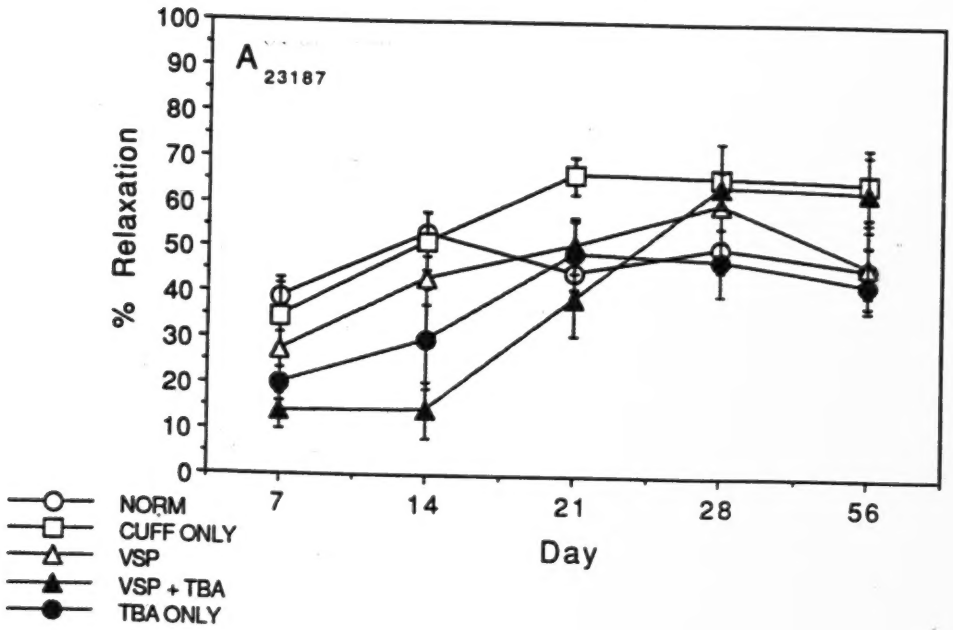

Figure IV-5

Responses on each study day of the five vessel catagories (normal control [NORM], cuffed non-blood coated [CUFF ONLY], cuffed blood coated [VSP], cuffed blood coated dilated [VSP+TBA], and uncuffed dilated [TBA ONLY]) to the endothelium-dependent vasorelaxant calcium ionophore A_{23187}. Percent relaxation was calculated using tonic contraction with 10^{-5} noradrenaline as 0% relaxation. Although concentration response curves were determined, only the response to the highest concentration of calcium ionophore (10^{-5} mol/L) on each study day is shown. The relaxations of normal control vessels and cuffed non-blood coated vessels were not significantly different on any day when tested with the calcium ionophore. The relaxations of cuffed blood coated undilated (vasospastic) vessels in comparison to normal control vessels were decreased on days 7 and 14, and slightly increased on days 21, 28, and 56 but the results did not reach statistical significance on any day. The relaxations of cuffed blood coated dilated vessels in comparison to normal control vessels were significantly diminished on days 7 and 14 but not on days 21, 28, and 56. The relaxations of uncuffed dilated vessels in comparison to normal control vessels were diminished on every study day except day 21, but not at a significant level.

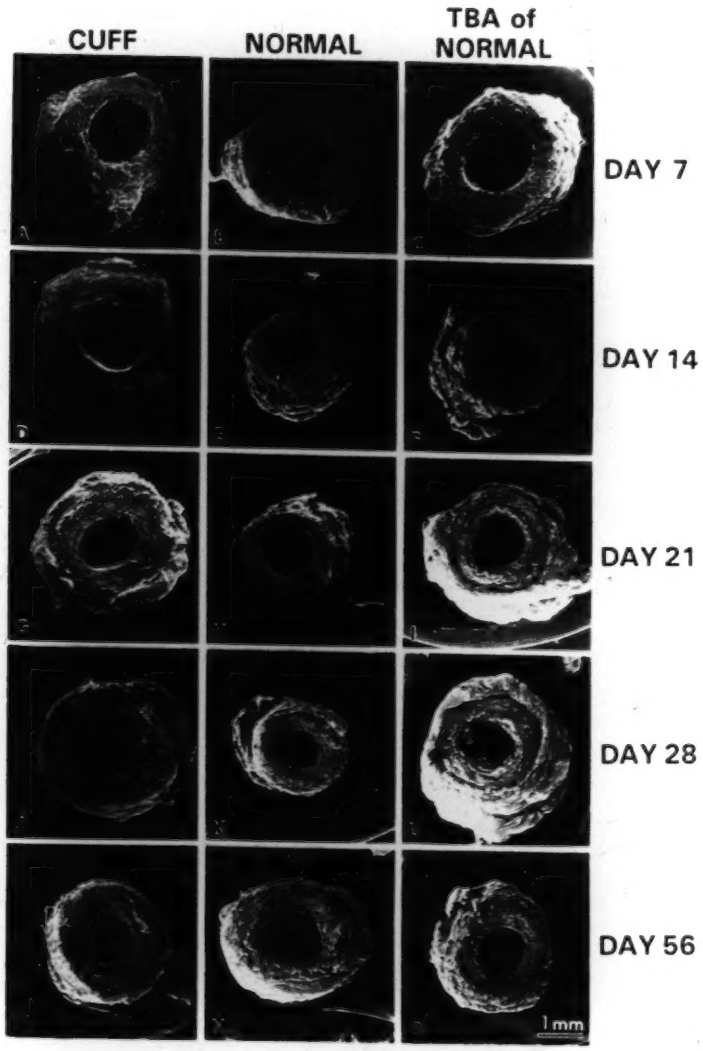

Figure IV-6
Scanning electron micrographs show similarities and differences between three of the vessel catagories on each of the study days. A,D,G,J,M, cuffed non-blood coated vessel segments (column labelled CUFF). B,E,H,K,N, normal control vessel segments (column labelled NORMAL). C,F,I,L,O, uncuffed dilated vessel segments (column labelled TBA of NORMAL). The presence of an empty cuff around the the artery for 7 days did not alter the appearence of the vessel wall (A compared to B): lumen diameter is not decreased, luminal/wall diameter thickness ratio is not decreased and there is no alteration of the internal elastic lamina or endothelial surface. The similarity between normal control and cuffed non-blood coated arteries was also present on the other study days. Dilatation of uncuffed vessels on day 7 resulted in slight enlargement of the vessel lumen above normal size, with some patchy endothelial denudation and straightening and thinning of the IEL (C compared to B). These distinguishing features were less notable on day 14 (F compared to E); on days 21, 28, and 56 they were almost absent.

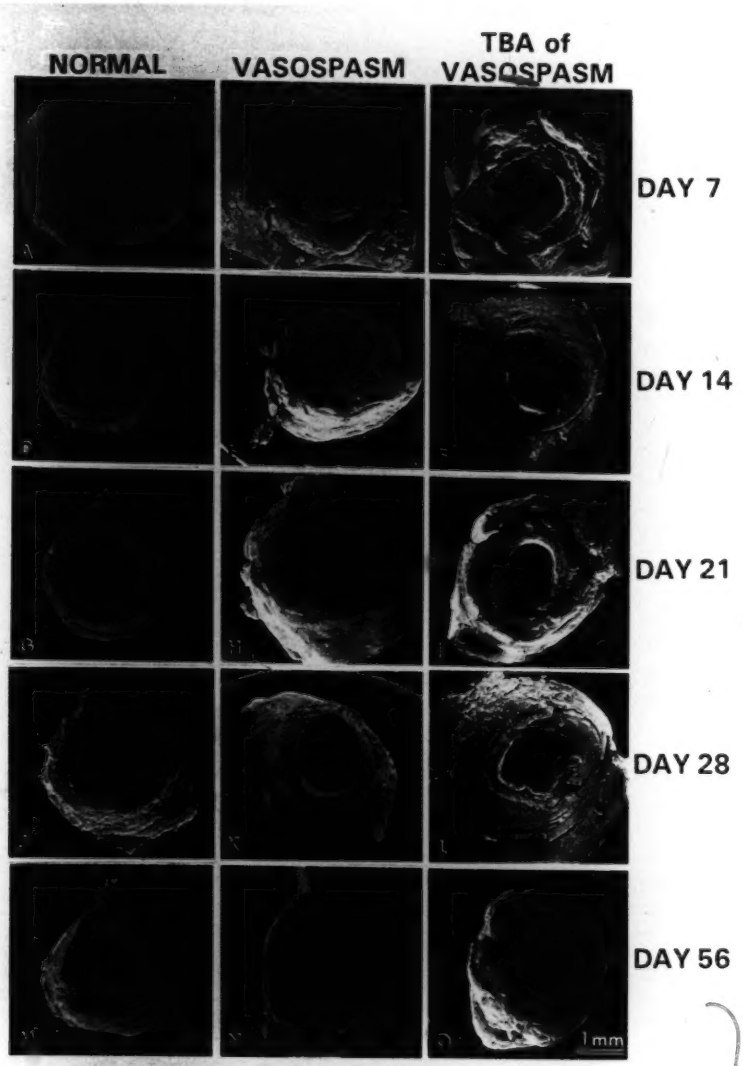

Figure IV-7
Scanning electron micrographs show differences between three of the vessel catagories on each of the study days. A,D,G,J,M, normal control vessel segments (column labelled NORMAL). B,E,H,K,N, cuffed blood coated undilated (vasospastic) vessels (column labelled VASOSPASM). C,F,I,L,O, cuffed blood coated dilated vessel segments (column labelled TBA of VASOSPASM). The presence of a blood filled cuff around the the artery for 7 days altered the appearence of the vessel wall (A compared to B): smaller lumen, decreased luminal/wall diameter thickness ratio, corrugation of the internal elastic lamina, and folding of the endothelial surface. The differences were still prominent on day 14 (E compared to D), less notable on days 21 and 28, and almost absent on day 56. Dilatation of vasospastic vessels on day 7 resulted in an enlargement of the vessel lumen to near normal size, with some patchy endothelial denudation and straightening and thinning of the IEL (C compared to B and A). The differences were still prominent on day 14 (F compared to E and D), less notable on days 21 and 28, and almost absent on day 56.

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

Canine high cervical internal carotid artery model of vasospasm

Conclusions

Over fifty models of subarachnoid hemorrhage (SAH) and vasospasm were reviewed. These models used one of two techniques to simulate SAH: (1) an artery was punctured allowing blood to escape and collect around the artery and its neighbors, or (2) an artery was surgically exposed and autologous blood obtained from another site was placed around the artery. Each of these techniques has advantages and disadvantages. Arterial puncture is more difficult to perform, but reproduces the physiological conditions associated with vessel wall rupture and direct bleeding into the subarachnoid space seen with aneurysmal rupture. Direct clot placement is easier to perform but in some models requires arterial manipulation. The model of SAH and vasospasm used most frequently is the canine "two hemorrhage" model, in which two injections of blood into the dog's basal cistern performed 48 hours apart result in greater arterial vasoconstriction than a single injection of blood. Based on its ability to accurately predict what occurs in human SAH, the best model of vasospasm appears to be the primate model in which blood clot is surgically placed around the large cerebral vessels at the base of the monkey's brain.

The majority of animal models of SAH and vasospasm use intracranial arteries, however recently extracranial arteries have also been used in vasospasm research. The experiments presented here show that the canine high cervical internal carotid artery (ICA) is suitable for studies on vasospasm. Placement of a blood clot-filled silicone elastomer cuff around the ICA resulted in angiographic arterial narrowing in 7 days. Arteries had pharmacological and morphological characteristics consistent with vasospasm. Placement of an empty cuff around the ICA had no significant effect on the artery. The angiographic vasospasm was still significant on day 14 but had resolved by day 21, which follows a time course consistent with vasospasm seen in humans. The pharmacological and morphological characteristics of these arteries also normalized over a similar time frame.

Recommendations

The canine high cervical ICA is readily exposed with no special surgical skills required. This is unlike many intracranial models of vasospasm which may require a craniotomy and manipulation of the brain to access the arteries at the skull base. Both ICAs are accessible through a single opening and this allows paired experiments with internal controls to be easily performed. The artery is approximately the same size as the human intracranial arteries commonly affected by vasospasm (the internal carotid artery and M1 segment of the middle cerebral artery). It is suitable for endovascular procedures using catheter balloon systems designed for humans. The cost of experiments might be considered as moderate, certainly less expensive than vasospasm studies performed in primates, but still more expensive than vasospasm experiments in rabbits or rats.

The obvious disadvantage of the model is that it uses extracranial arteries. There are definite pharmacological and morphological differences between extracranial and intracranial arteries, although the significance of these as they pertain to vasospasm research is uncertain. Overall the evidence presented in these studies indicates that the canine high cervical internal carotid artery model of vasospasm is a good one.

Immediate effects of TBA and its ability to prevent vasospasm

Conclusions

Arteries subjected to TBA and then surrounded by a blood clot-filled silicone elastomer cuff did not develop angiographic vasospasm in 7 days. The arteries exhibited altered smooth muscle cell function as determined by pharmacological analysis and some morphological changes, but no frank vessel wall disruptions. The exact mechanism responsible for the pharmacological impairment seen immediately after TBA cannot be determined from these experiments. It may be that TBA disturbs the smooth muscle cell membrane, disrupting both voltage gated calcium channels and membrane receptors, rendering the cell less responsive to agents that act at these sites. It may be that TBA alters endothelial cell function, leading to a reduction in nitric oxide synthesis, or that it alters the responsiveness of smooth muscle cells to nitric oxide.

Recommendations

More experiments are required to determine the method of action of TBA. It would be interesting to culture smooth muscle cells after TBA and try and determine if any of the proteins involved in smooth muscle contraction are altered. Since TBA is likely to effect the cell membrane, calcium channels and protein receptor complexes would be of particular interest. The role of the endothelium could be studied using the canine high cervical internal carotid artery of model. Endothelial cells could be mechanically removed from the inner surface of the artery to see if this alters vasospasm development and the effectiveness of TBA.

The ability of TBA to prevent angiographic vasospasm is somewhat tantalizing. There is already clinical evidence that TBA performed early in the course of clinical vasospasm is optimal for patient outcome. Some even feel that TBA of vasospastic vessels that are not apparently causing symptoms, (especially if one is "already there" dilating constricted vessels that are leading to symptoms) is desirable. Although experimental data are often useful in guiding clinical decision making, it is probably premature to recommend "preemptive" or "preventive" TBA based on this study alone (even though this was suggested in an editorial comment (1) on this aspect of the study (2)). Similar experiments to the one conducted here using larger numbers of animals and perhaps in one or two other animal models of vasospasm (preferably an intracranial primate model) should be done first. Then, if results are still positive a human trial might be considered.

Long-term effects of TBA

Conclusions

Vasospastic arteries subjected to TBA showed an immediate angiographic dilatation that was durable. When tested with pharmacologic agents these arteries exhibited a functional impairment of the vessel wall that was significant on days 7, 14, and 21, but not on days 28 and 56. Morphological alterations were present on days 7 and 14, resolving by days 21 and 28, and almost absent by day 56. Normal arteries undergoing TBA showed an immediate angiographic dilatation and functional impairment

of the vessel wall on days 7 and 14, but not on days 21, 28, and 56. Morphological alterations were present on day 7, resolving by day 14, and almost absent by days 21, 28, and 56. From these results it can be concluded that the functional and morphological alterations caused by TBA are not permanent. It may be that after TBA the artery consists of different cell populations, some of which may be approximately normal in function, while others have a gross impairment of function. Over the ensuing days cellular recovery occurs with the relative amount of normal to abnormal cells increasing until after three weeks the cell population is normalized to pre-TBA levels. It is unclear why the effect of TBA on normal vessels is not as marked as it is on vasospastic vessels and why the alterations in normal vessels resolve more quickly that similar alterations in vasospastic vessels after TBA.

Recommendations

More experiments are required to determine how arteries subjected to TBA recover. Smooth muscle cells cultured after TBA could be labelled and followed over time to see if certain subpopulations exhibit different proliferation indices. This might help to determine if some smooth muscle cells undergo phenotypic changes after TBA and if so how they revert to their pre-TBA state.

The clinical use of TBA is already supported by good long-term results in relatively large patient series. By extrapolation from the experimental results presented here, TBA as it is now performed in the clinical setting probably does not result in permanent alterations to the arterial wall. This may provide additional reassurance to those clinicians performing the technique.

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